PROTOZOOLOGY

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A MANUAL

FOR MEDICAL MEN, VETERINARIANS AND ZOOLOGISTS

BY

C. M. WENYON

C.M.G., C.B.E., M.B., B.S., B.Sc. (LOND.)

DIRECTOR-IN-CHIEF OF THE WELLCOME BUREAU OF SCIENTIFIC RESEARCH

FORMERLY PROTOZOOLOGIST TO THE LONDON SCHOOL OF TROPICAL MEDICINE

IN TWO VOLUMES

WITH 565 ILLUSTRATIONS AND 20 COLOURED PLATES

VOL. I

LONDON

BAILLIÈRE, TINDALL AND COX

8, HENRIETTA STREET, COVENT GARDEN, W.C.2

1926

PRINTED IN GREAT BRITAIN

To

FÉLIX MESNIL

MY FORMER TEACHER

THIS BOOK IS DEDICATED AS A TOKEN OF
PERSONAL INDEBTEDNESS AND RESPECT FOR
HIS MANY VALUABLE CONTRIBUTIONS
TO OUR KNOWLEDGE OF
PARASITIC PROTOZOA

CORRIGENDA

Page 41, Fig. 23, for *Cothurina* read *Cothurinia.*
,, 276, Fig. 125, for *hirudinella* read *hirundinella.*
,, 323, line 14, for *raiæ* read *rajæ.*
,, 474, line 2, for *hirudinis* read *hirundinis.*
,, 475, line 4 from bottom, for *hirudinis* read *hirundinis.*
,, 588, line 1, for *Sternotherus* read *Sternothærus.*
,, 604, Fig. 247, for *raiæ* read *rajæ.*
,, 710, line 19, for *Atelus* read *Ateles.*
,, 722, inscription to Fig. 307, line 7, for *spherulosa* read *sphærulosa.*
,, 806, line 29, for *aspic* read *aspis.*
,, 983, line 5, for 25-30 read 26-30.
,, 1116, Plate XX., for Sporozoan read Sporozoon.

PREFACE

THE subject of Protozoology has, in recent years, shown a tendency to become divided into two sections. In the one the student's attention is directed chiefly to the study of free-living Protozoa, in the other to parasitic forms, more especially those which give rise to disease in man and domestic animals. Such a division, if it becomes absolute, cannot lead to a clear understanding of the group as a whole, for it is evident that without some knowledge of free-living Protozoa, from which they have been undoubtedly evolved, a wrong conception of parasitic forms will be obtained. As in other branches of science, specialization appears to be inevitable if any advance is to be made, but however specialized a student becomes, it is his duty to keep himself informed of any progress made outside his particular field. Anyone who wishes to make an intelligent study of parasitic Protozoa must be acquainted with the fundamental principles of general Protozoology, and, indeed, with those of general Zoology, Physiology, and even other sciences. This is merely another way of stating the well-recognized fact that all sciences are interdependent. On this account the student of the Protozoa which are pathogenic to man and domestic animals should have a sound knowledge of other parasitic Protozoa, and at least a good working knowledge of non-parasitic forms as well. Conversely, those who study free-living Protozoa should have a clear conception of the parasitic forms, for the extensive investigations of recent years have contributed so much to our knowledge that in many respects they are better known than their free-living relations, particularly as regards the completeness of their life-histories and the probable course of their evolution.

In this manual the writer has attempted to present the subject of Protozoology in such a light that it will be of use to the zoologist who wishes to obtain information regarding the general principles of the subject and detailed knowledge of parasitic forms, and to medical men and veterinarians who are chiefly concerned with those Protozoa with which they have professionally to deal.

The investigations of Smith and Kilborne on the parasite of Texas fever of cattle and its transmission by ticks; those of Laveran, Golgi, Ross, and Grassi on malarial parasites of man and birds, and their carriage by mosquitoes; and the researches of Bruce, who demonstrated the

trypanosome nature of the African cattle disease, nagana, and its conveyance by tsetse flies, opened an entirely new field of enquiry which has led to a most extensive study of parasitic Protozoa. The thousands of papers on the subject which have been published during the past twenty or thirty years are scattered in numerous journals, many of which are difficult to obtain by any worker, and impossible by those who are stationed in parts of the world where good libraries are not available. Many workers have spoken to the writer of the difficulties associated with this separation from literature, and it has been largely a desire to remove at least a part of these difficulties that has led him to undertake the present work on the subject of Protozoology.

The book deals with all groups of parasitic Protozoa, as well as with free-living forms, though the latter have been dealt with very briefly, except in the case of those which are coprozoic in habit and may lead to confusion with parasitic organisms. The part played by invertebrates in the transmission of certain parasitic Protozoa of vertebrates necessitates the examination of invertebrates in order to trace the life-history of any parasite which may develop in them. As knowledge of the parasites which are peculiar to these invertebrates is essential if errors are to be avoided, they have accordingly received special attention.

In reviewing the extensive literature on the subject of Protozoology it has been necessary to criticize many statements and claims which have been made, but, in expressing his own views, the writer hopes that he has explained clearly the reasons which have led him to their adoption, and that he has treated fairly those records which appear to him to be of doubtful value.

One of the chief difficulties associated with the production of a manual like the present one is that hardly a week passes without the publication of some paper of importance; but an earnest endeavour has been made to incorporate all new and essential data as they appeared, so that as the book goes to press in its final form a fair claim can be made that it is as complete as it reasonably can be. Rapid advances are being made in the elucidation of the methods of transmission of kala azar and Oriental sore, and there is every prospect that very soon the sand fly will be incriminated definitely as the vector of one or both of these diseases. The treatment of general paralysis by inducing in patients attacks of malaria is leading indirectly to the discovery of many interesting facts regarding the development of malarial parasites. The recently described method of cultivation of intestinal amœbæ is assisting in the solution of many problems connected with the life-history of these organisms. Three hitherto supposed coccidia of man have been shown to be nothing more than parasites of edible fish which are passing casually through the human

intestine. Observations such as these are continually producing changes in our outlook, so that, however quickly a book is produced, it is bound to be out of date in certain respects when it appears. Nevertheless, the greater part of the information which will be found on its pages is well established, and will be lasting, so that it is sincerely hoped that the two volumes will provide a reliable record of our knowledge up to the beginning of 1926.

As the study of spirochætes is intimately related to that of the Protozoa, especially in connection with blood work, a section is devoted to their consideration, though it is definitely maintained that they are not Protozoa.

Many Protozoa which have affinities with those which produce diseases in man and domestic animals have been found in the blood of other vertebrates and in the intestines of invertebrates. A worker who discovers such an organism has considerable difficulty in ascertaining if it has been previously noted. To meet this difficulty a host list of the blood-inhabiting parasites of vertebrates and one of the flagellates of invertebrates have been compiled, and it is hoped they will be useful references.

As difficulties associated with nomenclature, the accuracy of which is of such importance, are constantly occurring, the International Rules of Zoological Nomenclature, which many workers have little opportunity of consulting, have been included.

The practical side of Protozoology has been constantly kept in mind, as well as the difficulties which beset the path of those engaged in its study. A special section deals with methods of investigations. This is not intended to be a complete account, but merely a guide for the use of those who already have a working knowledge of laboratory technique.

Authorities for all statements made in the text have been given, and the exact references will be found in the list of publications at the end of the book. Practically all these have been consulted in the original, and with very few exceptions every reference has been verified. The greater part of this laborious work has been carried out by Miss I. M. Bellis, Librarian to the Wellcome Bureau of Scientific Research, whose knowledge of languages and scientific publications has been invaluable. The writer is glad to have this opportunity of acknowledging his indebtedness to her for the great care she has taken with this and many other parts of the work. The writer has constantly had the assistance of Mr. Cecil Hoare, Protozoologist to the Wellcome Bureau of Scientific Research. Many intricate questions have been discussed with him, and his sound judgment, together with his careful and critical reading of the proofs, has been a great asset. For many of the drawings, both

black and white and coloured, the writer is much indebted to Mr. B. Jobling, now on the staff of the Wellcome Bureau of Scientific Research. His knowledge of biology combined with his artistic skill has enabled accurate copies and many original drawings from preparations to be produced. The writer's thanks are also due to his sister, Miss M. G. Wenyon, Assistant Secretary to the Royal Society of Tropical Medicine and Hygiene, who has read carefully the final proofs, and has been a means of detecting errors which otherwise would have marred the pages.

The writer is indebted to Professor Nuttall, F.R.S., Quick Professor of Biology and Director of the Molteno Institute of Cambridge; Dr. A. G. Bagshawe, C.M.G., Director of the Bureau of Hygiene and Tropical Diseases; Professor Warrington Yorke of the Liverpool School of Tropical Medicine; Professor A. E. Boycott, F.R.S., of University College, London; Lieut.-Col. W. P. MacArthur, D.S.O., O.B.E., of the Royal Army Medical College; Dr. Keilin of the Molteno Institute, Cambridge; and the Councils of the Royal Society of Tropical Medicine and Hygiene and the Royal Institution for the loan of blocks. He is also indebted to Mr. Clifford Dobell, F.R.S., for the use of his original diagrams of *Aggregata eberthi* and his drawing of the cyst of *Balantidium coli*, and for permission to reproduce figures from his publications.

Much assistance has been derived from many of the books on Protozoology or one or other of its branches, particularly Doflein's *Lehrbuch der Protistenkunde*, Laveran and Mesnil's *Trypanosomes et Trypanosomiases*, Laveran's *Leishmanioses*, Minchin's *An Introduction to the Study of the Protozoa*, Dobell's *The Amœbæ Living in Man*, Dobell and O'Connor's *The Intestinal Protozoa of Man*, and many others; but of all the publications, apart from original articles, the careful reviews by Professor Mesnil which have appeared regularly in the *Bulletin de l'Institut Pasteur* since 1902, and those by various writers in the *Tropical Diseases Bulletin*, have been most helpful. Any worker who wishes to keep abreast of the times cannot do better than to read one or both of these excellent bulletins with regularity.

Finally, the writer wishes to express his thanks to Mr. N. B. Kinnear of the British Museum, Natural History, for the trouble he has taken in checking the host list of birds, and to all the many others who have been ever ready to give him valuable assistance.

C. M. W.

LONDON,
June, 1926.

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PART I

GENERAL DESCRIPTION OF THE PROTOZOA

ORGANIZATION AND LIFE-HISTORY OF THE PROTOZOA.

DURING the latter part of the seventeenth century Antoni van Leeuwenhoek (1632-1723), working with a simple microscope, investigated free-living Protozoa and studied the parasitic forms in the intestine of frogs. He also found that he himself was infected with one of these organisms, which, as Dobell (1920) has pointed out, was probably the well-known *Giardia intestinalis*. The great Dutch microscopist thus not only discovered free-living Protozoa, but was the first to study parasitic forms, and he can be justly regarded as the father of Protozoology and of its more specialized branch, Medical Protozoology. Since Leeuwenhoek's day an ever-increasing number of investigators, availing themselves of the experiences of those who had gone before them and of the steady improvement in the microscope, have brought to light an enormous assemblage of minute living creatures, many of which, like the bacteria, were quite beyond the scope of the simple magnifying apparatus used by Leeuwenhoek and other early workers. These minute organisms absorb nourishment and grow, and finally, as in higher animals, reproduce by detaching portions of their bodies to form those of their offspring, while any remaining portion dies. It may be that the entire body of the parent is used up in the production of progeny, or only a small portion of it, as in higher animals, but in either case, extending from parent to offspring, there is a continuity which entitles all living creatures to be regarded as immortal in that a portion at least of the living matter is handed on from one generation to another, unless accidental death prevents reproduction.

The fact that all the complex mechanisms of life are concentrated in these minute portions of living matter has led observers to seek in them an explanation of the phenomena of life in general. A single organism may be kept under observation for the whole of its individual existence, and the visible changes undergone by it during its life, which is terminated by its final production of offspring, may be actually followed under the microscope. It seems evident that beyond the scope of the microscope there exist organisms, or stages of development of visible organisms,

which cannot be seen—the ultra-microscopic viruses. Dark field illumination has done much to facilitate the study of these forms, but, as yet, the exact nature of the numerous minute objects which it has revealed in every fluid, and which are in constant motion (Brownian movement), has not been satisfactorily determined, so that at present it is in many cases impossible to decide whether they are actually living organisms or granules of inanimate material.

The study of microscopic organisms has revealed the fact that, in their method of nutrition, some of them resemble plants and others animals. On the basis of this physiological distinction it has been the custom to regard them as belonging to one of two main groups—the Protophyta and the Protozoa. The study of the former has been relegated to the botanist, and that of the latter to the zoologist. Though some of these organisms show undoubted affinities with the algæ and higher plants and others with animals, there exists a miscellaneous assemblage of indeterminate forms which cannot be placed legitimately in either group. Accordingly, it is safer to regard them all as belonging to one large group, the Protista, the study of which is known as Protistology, as first suggested by Haeckel (1866). Without being able to define accurately the limits of either group, it is nevertheless convenient to regard the Protista as comprising the two subdivisions of the Protozoa and the Protophyta. In the case of the former, nutrition is effected by the ingestion of preformed proteid material, either as solid particles or in solution. The Protophyta, on the other hand, nourish themselves like plants on comparatively simple chemical compounds, and when possessing chlorophyll or some similar substance, make use of the carbonic acid of the liquid in which they live. Very frequently they secrete around themselves capsules of cellulose. A typical Protist consists of a small portion of cytoplasm and a nucleus which contains as its most essential constituent a substance called chromatin. The contents of the nucleus are separated from the cytoplasm by a nuclear membrane. Other bodies may be present in the cytoplasm, but these, at least as definite visible structures, are not essential to life.

Amongst the existing Protista the most primitive forms are possibly the bacteria, spirochætes, and allied organisms, which in many cases do not appear to possess definitely constituted nuclei, though granules of a substance which some observers have identified with chromatin are present in the cytoplasm. Alexeieff (1924*a*) maintains that it is not chromatin, and that this substance is absent from bacteria. These forms, however, are in most cases so minute that accurate information regarding their cytological structure and life-histories is difficult to obtain. It can, at any rate, be safely affirmed that those Protista which are most

highly developed and most complex in structure possess definite nuclei, and the small particle of cytoplasm with its included nucleus of which the body of each is composed is regarded by most biologists as a cell on account of its resemblance to the cells of higher animals and plants.

The term "cell" was first introduced for the cellulose capsule or wall which encloses the portions of cytoplasm of which the higher plants are built up. It was later realized that the wall itself was merely a supporting structure, and that the cytoplasm within it was in reality the living material. Accordingly, the term "cell" was then applied, not to the cell wall, but to its cytoplasmic contents. The latter consists typically of a small mass of cytoplasm containing a single nucleus. When it was discovered that the tissues of higher animals were also built up of similar elements or units, the term "cell" was applied to them also. It soon became evident that, in the case of many microscopic organisms, the entire body consisted of a similar mass of cytoplasm containing a nucleus, and the resemblance of these to the cells of higher animals and plants gave rise to the view that these organisms were single cells, and the distinction between unicellular and multicellular animals was drawn. This conception, which was first clearly expounded by Schwann (1839), has been generally accepted, though Dobell (1911) considers it erroneous. He believes that an amœba, for instance, is as much an entire organism as one of the higher animals, and that though the latter may be regarded as being multicellular, as a result of the division of its cytoplasm and nucleus into cells, the former should be considered as a non-cellular organism, and not a unicellular one, since it corresponds, not to any single cell, but to all the cells which compose the body of the multicellular organism.

When it is realized that amongst the numerous cells which compose the body of one of these higher animals there are many wandering cells, such as macrophages, which behave in all essential respects like amœbæ, in that they form pseudopodia, ingest solid proteid material of various kinds, and multiply by fission, it is difficult to resist the conviction that such a cell has a definite claim to be regarded as an individual organism like an amœba itself. Furthermore, it has been clearly demonstrated that very minute portions of the tissues, consisting of groups of cells, or even single cells of higher animals, can be artificially cultivated, and that they will live and multiply indefinitely provided they are given a continuous supply of suitable nutriment. From these culture experiments it seems clear that the cell, which forms but a part of the entire multicellular animal, is capable of nourishing itself and reproducing as a single organism. Another illustration of the power of independent existence and multiplication of isolated cells of multicellular animals is seen in malignant disease. In this condition

certain cells acquire the power of continuous and rapid multiplication, so much so that they become to all intents and purposes parasites, which bring about the death of their host. These cells can be inoculated from animal to animal indefinitely, and in them they will continue to multiply, just as trypanosomes do in successive passages in experimental animals.

An ovum, according to the non-cellular view, is a non-cellular individual, which at once becomes cellular when segmentation occurs. The cells, each of which gives rise to only part of the individual which will normally develop from the ovum, are nevertheless potential individuals themselves, as is demonstrated by the fact that if the cells are separated from one another artificially, as in the well-known experiments with sea-urchin eggs, each is capable of giving rise to a complete embryo.

It seems evident that the cells of higher animals are capable of independent life provided the proper environment exists. Under natural conditions all the cells of the body contribute to the production of this environment, which is so delicately balanced that separated and isolated cells invariably die unless the proper environment is present or is artificially provided, as in the culture experiments just mentioned. If the environment necessary for the continued life of cells in the body can be kept constant, the cells will survive and reproduce indefinitely, but if some of the cells fail to fulfil their part in the production of this environment, the other cells will suffer and death will result. It may be said that any single cell of a Metazoon is living in a condition of symbiosis with all the other cells. Without entering further into the discussion, for purposes of this work it is sufficient to follow the more orthodox view and to regard the Protista as unicellular organisms, or single cells which still lead a completely independent existence, and the multicellular organisms as groups of cells which work together for a common end. The latter have become so completely interdependent that their power of separate existence has been largely lost. Yet in many features, such as their structure, mode of life and method of reproduction, nuclear division and syngamy, they retain the unmistakable characteristics of their unicellular ancestors. It must not be supposed that the ancestors of either the multicellular or unicellular organisms any longer exist. The primitive forms from which they may be supposed to have originated have probably long since disappeared in the course of evolution. The Protista of the present day, as well as the individual cells of higher animals and plants, have undoubtedly evolved along different lines and acquired certain characteristics which their common ancestors did not possess. Biologists are nevertheless justified in still regarding the portion of cytoplasm with its nucleus as a cell, whether it occurs amongst the Protista or the Metazoa and Metaphyta, in spite of the fact that the cells of each group may now

possess distinctive features of their own. The cell may be justly regarded as an individual, whether it is one of the Protista or only part of the body of a multicellular organism. In the latter case it must be admitted that a number of individuals have remained united as a colony to form a single larger individual. Of the cells of the latter, only certain ones are destined for reproduction, as in the case of spores of Cnidosporidia, where a group of cells is formed by division from a single cell, and of these only one is a reproductive cell, the others dying after fulfilling other functions. A single soldier or a regiment of soldiers may both be units in the military sense, but the soldiers composing the regiment, though sacrificing their individuality to some extent for the good of the individual regiment, are as much individuals as the single soldier.

It has been clearly demonstrated that a Protozoon quickly dies if deprived of its nucleus, and there is little doubt that the cells of higher animals are similarly dependent on their nuclei. A single unicellular organism may be divided into several portions, but though those which do not contain the nucleus may exhibit movements and survive for some time, they ultimately die, whereas any nucleated portion may re-form itself into an entire individual which is able to continue its existence. It is evident the nucleus plays a very important part in the life, and metabolism of the cell. The Protozoan cell does not differ from other cells in its capacity to absorb and digest food, and grow and increase in size. It is able to perform spontaneous movements as a result of contractions of its cytoplasm, though these are reduced to a minimum in some cases. Finally, the cell is able to multiply, usually by a process of *binary fission*, but sometimes by a process of *multiple fission*. In binary fission the single nucleus divides into two parts, and this is followed by division of the entire cell into two daughter cells. Usually, these are approximately equal in size (*equal binary fission*), but it may happen that one daughter individual is larger than the other (*unequal binary fission*). When the difference in size is marked, it appears as if a small daughter individual is separated from a much larger parent which retains its original form, and the process is spoken of as *budding* or *gemmation*. In the case of *multiple fission* or *multiple segmentation*, after the first division of the nucleus the body of the organism does not immediately divide, but the two daughter nuclei again divide to form four nuclei, and these may again divide to give rise to eight. After a number of nuclei have been thus produced by repeated divisions, the body of the organism segments into, or more accurately buds off, a number of portions corresponding to the number of nuclei. This method of multiple fission of cells, which more correctly should be called multiple gemmation, occurs in higher animals as well as in the Protozoa, amongst which it is seen

typically in the parasitic Sporozoa, and is known as *schizogony*. Usually there is a residue which does not participate in the formation of the buds; it is discarded as a *residual body* which quickly disintegrates.

During the life-history of many cells a sexual process occurs from time to time. The advantages gained from such a process, which is called *syngamy*, are far from being clearly understood. In its simplest form it consists in the complete union of two cells and fusion of their nuclei. The uniting cells are known as *gametes*, and the single cell resulting from the union is a *zygote*. The zygote proceeds to multiply by binary or multiple fission.

The process of syngamy must be distinguished from another type of union which sometimes occurs. Two or more cells may fuse to produce a multinucleate cytoplasmic body known as a *plasmodium*. In this

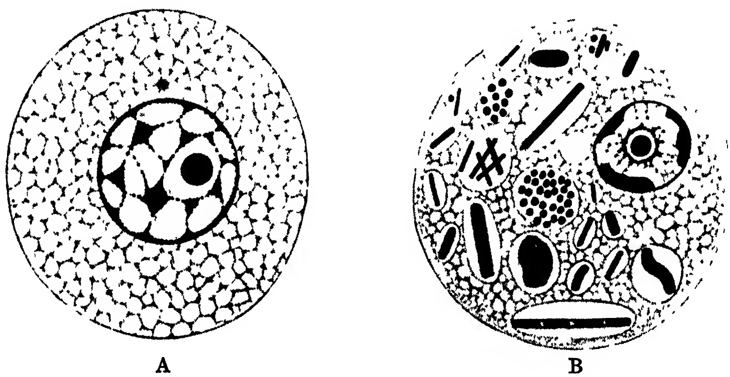


FIG. 1.—DIAGRAM OF CELLS. (ORIGINAL.)

A. Metazoan cell. The cytoplasm contains a centrosome and a nucleus with a nucleolus.
B. Protozoan cell (*Entamœba*). The cytoplasm, differentiated into ectoplasm and endoplasm, contains a nucleus with central karyosome and numerous food vacuoles. No centrosome is visible.

manner plasmodia containing many hundreds of nuclei may be formed. The nuclei show no tendency to unite with one another, as they do in syngamy, and after the plasmodial phase has existed for some time segmentation into uninucleate cells takes place.

The typical cell, wherever it occurs, consists of the two essential parts —cytoplasm and nucleus (Fig. 1). Each of these is a mixture of substances of highly complex chemical constitution, the reactions of which produce the phenomena characteristic of living matter. The cytoplasm appears to be made up of at least two substances, one of which is suspended in the other in the form of an emulsion. The nucleus, which is limited by a nuclear membrane, consists of a substance called nuclear sap, which occupies interstices in a more solid material. The latter, when viewed in

optical section, has the appearance of a network, and is known as the *linin network*, of which the nuclear membrane may be regarded as a special development. Upon this network, and on the nuclear membrane in the form of granules or larger masses, is arranged another substance, the *chromatin*, which has a strong affinity for certain stains. It is generally regarded as the most important constituent of the nucleus, and this is borne out by the fact that nuclear division takes place by an elaborate process known as *mitosis*, which results in an equal sharing of the chromatin between the daughter nuclei. In the nucleus of the Metazoan cell there is usually present a conspicuous body known as the nucleolus. It is devoid of chromatin, and when nuclear division takes place it passes to one of the daughter nuclei, the other daughter nucleus forming a new nucleolus. A very similar body exists in the nuclei of certain Protozoa (*Opalina*), and it passes to one of the daughter nuclei when division takes place. In other Protozoa, as, for instance, in *Karyolysus* and *Hepatozoon*, a similarly achromatic body divides at nuclear division, each daughter nucleus receiving half (Fig. 35). When such a body occupies a central position in a Protozoan nucleus it is known as a *karyosome*, and it has been generally assumed that it is composed largely of chromatin. It is becoming increasingly evident, however, that the karyosome may be actually devoid of chromatin, and the supposition that in certain nuclei the entire chromatin may be concentrated in the karyosome is a very doubtful one. The nucleus is often regarded as consisting of two substances—the achromatic and the chromatic material. The achromatic material, including the nuclear membrane, linin network, nuclear sap, and other bodies (karyosome, nucleolus) which are sometimes present, undoubtedly comprise several distinct substances, some of which, at any rate, are able to give rise to chromatin, for the quantity of chromatin in the nucleus varies from time to time, and increases with its growth. Another important constituent of the cell, which as a rule only becomes visible during nuclear division, is the centrosome (Fig. 1, A). It is commonly present in the cells of Metazoa, but it is not so frequently seen in the Protozoan cell. Reproduction of a cell by binary fission or multiple segmentation is always preceded by division of the centrosome, if one is present, followed by division of the nucleus, which in most cases takes place by mitosis. It is during nuclear division that the nature of many of the constituents of the nucleus first comes to light, and for this reason it will be necessary to consider mitosis, as it occurs typically in the Metazoan cell. During mitosis there are formed, mainly out of the chromatin, certain bodies known as *chromosomes*, which are constant in number for each species of animal, the same number appearing at each succeeding nuclear division. There is some evidence that in the resting, or more accurately the non-

dividing nucleus, though the chromosomes are no longer visible as individual units, they still exist as separate entities. During syngamy, when two gametes unite and their nuclei fuse, the chromosomes of the two uniting nuclei enter the zygote nucleus, so that, unless a reduction is made in the number of chromosomes, at each succeeding union the chromosome number would be doubled. Usually the number of chromosomes in the gamete nuclei is only half that of the nuclei of other cells of the body, and the process by which this reduction is brought about is known as the reducing division, or *meiosis*.

Though in the vast majority of cases it is recognized that the nuclei of daughter cells are the products of division of the nucleus of a parent cell, it is supposed that occasionally amongst the Protozoa nuclei may be formed from extra-nuclear chromatin granules which appear in the

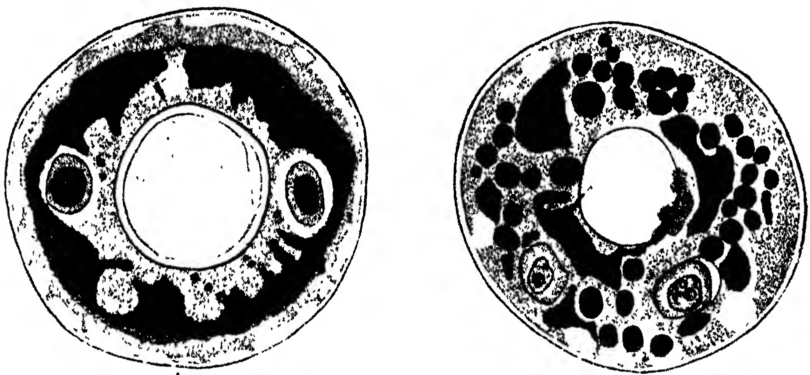


FIG. 2.—FORMATION OF NUCLEI FROM THE CHROMIDIAL BODY IN *Arcella vulgaris* (× *ca.* 300). (AFTER R. HERTWIG, 1899.)

A. Normal individual with two nuclei and mass of chromidial substance.
B. The chromidial substance is breaking up and nuclei are being formed from the fragments.

cytoplasm (Fig. 2). It seems to be an undoubted fact that chromatin material in the form of granules may leave the nucleus and take up a position in the cytoplasm. This has been described as taking place, not only in Metazoan cells, but also in the Protozoa. Such granules of chromatin, which occur in the cytoplasm, are known as *chromidia*. It is not, however, an easy matter to determine the true nature of granules which occur in the cytoplasm, and it has not infrequently happened that identical granules or material have been described as chromatin by one observer, and as some other substance by another. There seems little doubt that both in the case of Metazoan cells and Protozoa, chromidia do not occur so frequently as some have supposed. When the question of the origin of nuclei from these chromidia is considered there is still

greater uncertainty. Some observers believe that the chromatin granules or chromidia in the cytoplasm may, under certain conditions, arrange themselves in groups, each of which becomes transformed into a nucleus. It is difficult to avoid the impression that most, if not all, of the records of nuclei arising, as it were, by crystallization of chromidia are the result of misinterpretations, and that the appearances on which the conclusions have been based might be accounted for in another and more probable manner. In all cases in which accurate and continuous observation of reproducing cells has been possible, daughter nuclei have been found to arise only by division of pre-existing parent nuclei. A classical instance of this kind is seen in *Arcella vulgaris*, a binucleate shelled amœba (Figs. 2 and 79). Like many other shelled amœbæ, in addition to the true nuclei, *Arcella vulgaris* contains a mass of material which, on account of its affinity for certain chromatin stains, is supposed to be of chromidial nature, and is called the chromidial body. It was claimed by Richard Hertwig (1899) and other observers that at certain phases of development the two existing nuclei degenerate and disappear, and that numerous secondary nuclei are formed from the chromidial body. Schirch (1914) has, however, shown that in some cases, at least, the numerous nuclei which are present result from repeated divisions of the two which occur in the normal individual. It seems not improbable that the so-called chromidial body of *Arcella* and its allies is not really of chromatin nature, but consists of a special material which may be concerned with the development of the shell, which is a characteristic feature of these shelled amœbæ.

TYPICAL DIVISION OF THE METAZOAN NUCLEUS.

1. Mitotic Division.

The Protozoan nuclei divide in a variety of ways, and it is probable that amongst them the more primitive types of nuclear division will be found. There is every transition between what is little more than a simple constriction of the nucleus into two parts (*amitotic division*) and the elaborate method of division known as *mitosis* or *karyokinesis*, in which chromosomes are formed and divided in such a manner that the chromatin of the nucleus is equally distributed to the daughter nuclei. The division of nuclei by mitosis occurs most typically in the cells of higher animals and plants, and it was in their cells that the details of the process were first elucidated. The terms employed for the different structures and the various stages which occur were first applied to their nuclei, and were used subsequently for the corresponding stages which occur during the division of Protozoan nuclei. Mitosis in its typical form is characterized by the formation from the chromatin and achromatic material of the nucleus of a number

of usually elongate structures called chromosomes, each of which splits longitudinally into two daughter chromosomes, one of which passes into each daughter nucleus. This division and separation of chromosomes is associated with the formation of the *achromatic figure* which arises in connection with a structure called the *centrosome* situated in the cytoplasm outside the nucleus. The whole process can be regarded as taking place in a number of stages known as the *prophase*, *metaphase*, *anaphase*, and *telophase* (Fig. 3).

PROPHASE.—The centrosome, which is a spherical structure at the centre of which is a deeply staining granule, the *centriole*, divides into two parts which separate from one another. As they separate, the two daughter centrosomes remain connected by fibres which are arranged as a spindle, the *spindle fibres*, while similar fibres radiate into the cytoplasm from the centrosomes (Fig. 3, B and C). Each centrosome with its radiating fibres constitutes the *aster*. Within the nucleus the linin network becomes arranged in what has been supposed to be a long coiled thread in which the chromatin granules are embedded. This thread is known as the *spireme*. Structures such as nucleoli and karyosomes may break up and disappear, and any chromatin they contain becomes arranged in granular form with the rest of the chromatin of the nucleus upon the spireme. Finally, the nuclear membrane disappears, while the spireme segments into a number of chromosomes (Fig. 3, C). It seems probable that the conception of the spireme as a single long coiled thread is not correct, and that from its first appearance it consists of a number of long, intercoiled, separate segments which become distinct as they contract to form the *chromosomes*, the name given to the separate parts into which the spireme was supposed to divide. With disappearance of the nuclear membrane the separate chromosomes, each of which can often be seen to consist of two closely united parallel threads, arrange themselves in a looped fashion round the equator of the spindle, and in the plane of this equator in such a manner that the bend of each loop is directed towards the centre and the two ends away from it (Fig. 3, D and E). The chromosomes, which have become shorter and thicker at the equator of the spindle, form the *equatorial plate.*

METAPHASE—The chromosomes, which are now arranged as the equatorial plate, and each of which may consist of two closely apposed parallel structures, divide longitudinally into daughter chromosomes, which commence to move towards the pole of the spindle (Fig. 3, E).

ANAPHASE.—The daughter chromosomes now separate completely into two groups at the poles of the spindle. The natural interpretation that they are drawn there by the action of the fibres of the spindle to which

they are attached does not appear to be a satisfactory explanation of their movements (Fig. 3, F).

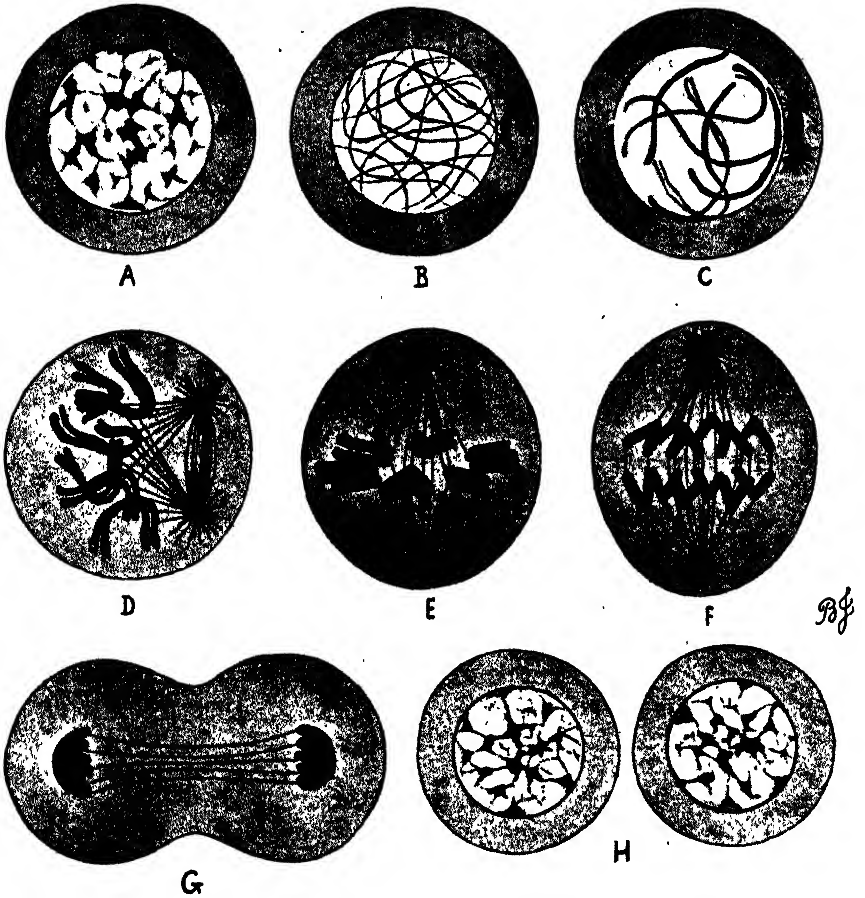


FIG. 3.—DIAGRAM OF NUCLEAR DIVISION BY MITOSIS. (AFTER AGAR, 1920.)

A. Resting nucleus with centrosome.
B. Early prophase with dividing centrosome.
C. Middle stage of prophase. appearance of spindle and dividing chromosomes.
D. Late prophase.
E. Metaphase with divided chromosomes as equatorial plate.
F. Anaphase: separation of daughter chromosomes.
G. Telophase: aggregation of chromosomes.
H. Completion of nuclear and cell division and reconstructed daughter nuclei.

TELOPHASE.—The spindle fibres gradually disappear, the nuclear membrane re-forms around the chromosomes, which gradually become transformed into the linin network and chromatin characteristic of the

original nucleus (Fig. 3, G and H). The centrosome remains outside the nucleus, the fibres of the aster becoming no longer visible.

The centrosome appears to be the ruling factor in the process, and the appearance of the aster and spindle fibres can be interpreted as visible indications of some force which is being exerted. It must be remembered, however, that in the mitotic division of the nuclei of the higher plants, as also that of many Protozoa, though all the stages of mitosis seen in the animal cell occur, definite visible centrosomes are not present. The fibres of the aster and spindle radiate from an apparently structureless area, which may be regarded as a potential centrosome. An important fact to be noted is that for any particular species the number of chromosomes present in the nucleus of any cell of the body is constant. In the much studied cells of *Ascaris megalocephala*, of which there are two varieties, the number of chromosomes is two or four respectively. In man it is twenty-two, while in other animals it may be much higher than this. Each species of animal has thus a definite chromosome number.

The chromosomes which are formed in any nucleus are not necessarily all alike in size or form. It is often found that they can be grouped in pairs, the members of each pair resembling one another more closely than those of other pairs. The members of each pair are known as *homologous chromosomes*. During the progress of mitotic division the chromosomes are at first elongate structures, but there is a tendency for them to shorten, so that at the stage when the equatorial plate is formed they may be roughly spherical. Though these alterations in size take place, all the chromosomes are similarly affected. Their relative size and shape remain the same, so that the homologous pairs can still be recognized.

During the telophase, when the chromosomes of the daughter nuclei are becoming transformed to reproduce the structure of the resting nucleus, it can sometimes be seen that the chromatin and achromatic material of each chromosome is occupied in reconstructing a particular portion of the nucleus. When chromosomes are re-formed at the next nuclear division, the material in each portion concentrates again into a chromosome. In these cases it appears as if there is a permanent separation of the constituents of each chromosome, even when the nucleus is in the resting condition. This has given rise to the doctrine of the *continuity of chromosomes*, which supposes that each chromosome is a permanent structure, which, though changing its form, is present as an individual unit even during the period when the nucleus is not dividing. The proof of this, however, is exceedingly difficult to obtain, and it must be regarded at present as little more than a plausible theory.

The chromosomes themselves are not homogeneous bodies, but consist

of a number of small granules of chromatin of varying size, the *chromomeres*, embedded in an achromatic matrix. Very frequently homologous chromosomes resemble one another very closely as regards the arrangement and variations in size of the chromomeres which they contain.

2. Meiotic or Reducing Division.

A sexual process or syngamy, which consists in the union of two cells together with fusion of their nuclei, occurs in higher animals and plants, and it was amongst them that the nuclear changes associated with the process were first studied. Attention has been drawn to the fact that the chromosome number for each individual species is constant, so that it must be evident that, if the nuclei of two cells unite, the number of chromosomes in the resulting zygote nucleus, which is known as the *synkarion*, would be double the usual number. This increase in number does not actually occur, for the nuclei of the uniting cells or gametes contain only half the number of chromosomes possessed by other cells. The reduction is brought about by a special type of mitotic division of the nucleus during the formation of the gametes (Fig. 4). When the chromosomes arrange themselves on the spindle fibres as the equatorial plate, instead of splitting into daughter chromosomes as in ordinary mitosis, they become separated into two groups, one of each pair of homologous chromosomes passing to each group (Fig. 4, C and D). In this way the daughter nuclei contain half the number of chromosomes possessed by the parent nucleus. The reduction in the number of chromosomes in the nuclei of the gametes is effected either at the last cell division which gives rise to gametes, or at the one immediately preceding it. The process is known as *meiosis,* and the nuclear division the *meiotic division* or *reducing division.* When the gametes unite and their nuclei fuse, the synkarion therefore contains the usual number of chromosomes. The gamete with half the number of chromosomes is said to be *haploid* as regards its chromosomes, while the original cell from which the gametes were derived and the zygote resulting from their union, which contain both chromosomes of each homologous pair, are said to be *diploid.*

Amongst the higher animals, as also frequently amongst the Protozoa, the gametes can be distinguished as male and female. The former, in the vast majority of cases, are smaller than the latter, so that the gametes can be distinguished as *microgametes* and *macrogametes.* The microgamete of a Metazoon is known as a spermatozoon and the macrogamete as an ovum. The microgametes are derived from a large number of cells called spermatogonia, which, like all the other cells of the body, contain the normal or diploid number of chromosomes. One of these cells in-

creases in size and becomes the *primary spermatocyte.* By division two *secondary spermatocytes* are produced, and each of these again divides, giving rise to four *spermatids,* which become directly transformed into microgametes or spermatozoa. It is during the first or second of these two divisions that meiosis occurs and the number of chromosomes is reduced. When it occurs it is seen that, as the chromosomes arrange

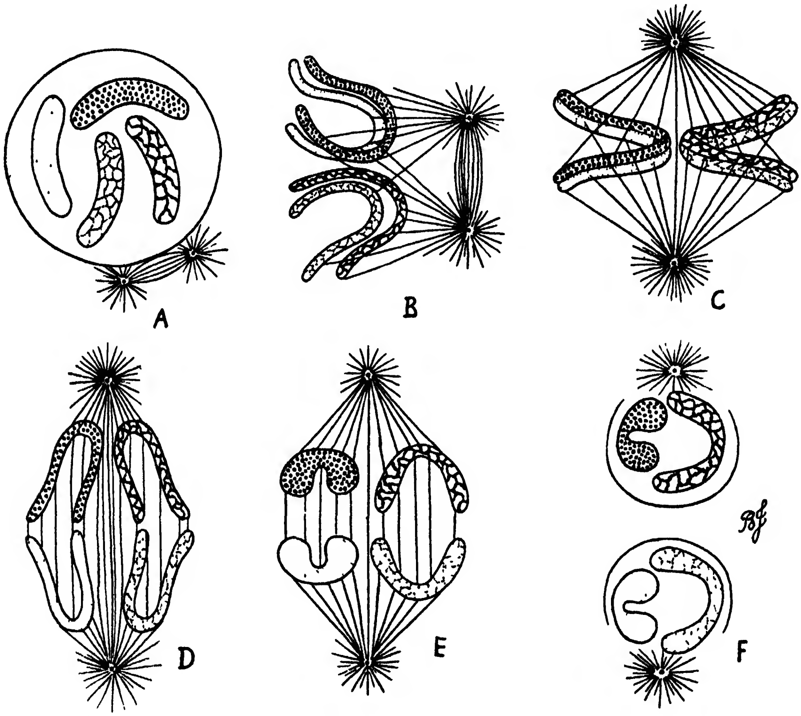


FIG. 4.—DIAGRAM OF MEIOSIS OR REDUCING DIVISION OF A NUCLEUS WITH FOUR CHROMOSOMES. (ORIGINAL.)

A. Showing two pairs (dotted and lined) of homologous chromosomes and commencing formation of spindle.
B and C. Syndesis or conjugation of homologous chromosomes.
D and E. Separation of the conjugated homologous chromosomes.
F. Formation of nuclei, each with half the original number of chromosomes; one of each pair of homologous chromosomes has entered each nucleus.
In ordinary mitosis the chromosomes at C, instead of separating, would divide, so that two pairs of homologous chromosomes would pass to each daughter nucleus.

themselves at the equator of the spindle, the individuals of each pair of homologous chromosomes are closely applied to one another, so that at first inspection it might be thought that only half the number were present. This approximation of the chromosomes of each pair is known as the *conjugation of the chromosomes* or *syndesis,* and it is supposed that

exchange of material takes place between them. As division of the nucleus proceeds, separation of the conjugating chromosomes occurs, and the two chromosomes of each pair pass to opposite poles of the spindle. It will be seen, therefore, that in this division there has been no splitting of the individual chromosomes as occurs in ordinary mitosis, but merely a separation of two chromosomes which have come together temporarily in syndesis. The number of chromosomes in the daughter nuclei are thus half the original number. If the reduction division occurs at the division of the primary spermatocyte, then the division of the nucleus of the secondary spermatocyte is not a reducing one, the chromosomes splitting longitudinally in the usual manner, so that the number is maintained. If the reduction occurs at the division of the secondary spermatocyte, then the division of the nucleus of the primary spermatocyte is of the ordinary type. In any case, the spermatids which become spermatozoa or gametes have half or the haploid number of chromosomes.

In the case of the female cell similar changes occur. Cells called *oögonia* grow into primary *oöcytes.* A primary oöcyte divides to give rise to two cells, which are, however, unequal in size. The large one is the *secondary oöcyte*, and the small one the *first polar body.* The secondary oöcyte divides into two cells, which are again unequal in size. One of these is the *ovum*, and the other the *second polar body.* The first polar body, which corresponds to a secondary oöcyte, may itself divide into two cells. The nuclear changes which occur in these divisions are similar to those which occur during the divisions of the spermatocytes described above, so that the number of chromosomes in the ovum is half the number present in the oögonia. There is this difference, however: Whereas each primary spermatocyte gives rise to four spermatozoa, each primary oöcyte gives rise to one large ovum and two small polar bodies, or three if the first polar body divides. By this arrangement the cytoplasmic part of the ovum is increased at the expense of that of the polar bodies, which do not proceed further to develop. Another difference between the ovum and spermatozoon is that the centrosome of the ovum has disappeared, though that of the latter has persisted.

When conjugation or syngamy occurs, the nucleus of the microgamete or spermatozoon unites with that of the macrogamete or ovum by a process known as *karyogamy* to produce the nucleus or *synkarion* of the zygote, which again has the diploid number of chromosomes arranged in homologous pairs. The centrosome of the microgamete becomes the centrosome of the zygote.

The pairs of homologous chromosomes of the zygote can be recognized through all the subsequent divisions of the cell down to the moment when the new adult individual again produces spermatocytes or oöcytes.

One chromosome of each pair was originally derived from the spermatozoon and the other from the ovum, and the two, or at least their descendants, have remained distinct during all the subsequent divisions of the nuclei. When the reducing division or meiosis occurs, the conjugation of the individuals of each pair of chromosomes takes place, and it is supposed that at this moment there is interchange of material between them, and that transmission of hereditary characters is accomplished.

It will be shown below that amongst the Protozoa the production of gametes may be associated with similar changes in the nuclei, the gametes possessing half or the haploid number of chromosomes. On the other hand, cases are known in which no reduction in the number of chromosomes takes place during gamete formation. It results that the zygote contains double or the diploid number of chromosomes. In these cases the first division of the zygote nucleus is a reducing division, the two daughter nuclei again having the haploid number. In the one case the reduction affects the gametes and occurs before syngamy, while in the other it affects the two daughter cells, resulting from division of the zygote, and occurs after syngamy.

GENERAL MORPHOLOGY OF THE PROTOZOA.

Of the Protozoa there are a very large number of genera and species, some of which are free-living forms, while others lead a protected existence within the bodies of higher animals. The latter have undoubtedly been derived from the former, and have become modified to such an extent in adaptation to their hosts that, generally speaking, they are no longer able to live apart from them. As practically every higher animal is liable to harbour in its body one or more Protozoa, it is evident that the number of parasitic species is very large indeed. It should be remembered, however, that to understand properly the parasitic forms the study of the free-living Protozoa should not be neglected.

It is customary to regard parasites in general as degenerate organisms, but though it is true they may have lost many of the organs possessed by their free-living ancestors, they may have developed others in their place, and reveal the same degree of adaptation to their environment as free-living forms. Though a parasite may have lost certain structures which it no longer requires, it digests its food, grows, and reproduces with all the complexity exhibited by those which still possess them. It seems incorrect to regard as in any sense degenerate an organism which is so completely adapted to its environment as are the majority of parasites. In fact it might be legitimately argued that if an organism retained structures for which it had no further use, this would indicate a loss of

adaptability to environment which in itself should be regarded as a sign of degeneration.

SHAPE AND SIZE OF THE BODY.—The Protozoa vary considerably in size, some of them being easily detected with the naked eye. Many of the ciliates and gregarines can be seen as white specks or elongate filaments, while certain multinucleate amœboid organisms may be several centimetres in diameter. The majority of Protozoa, however, are so small that they cannot be seen without magnification. The adult individuals of any species may vary considerably in size amongst themselves, and there may be marked differences in size between the mature and immature stages of development.

Protozoa may be of almost any conceivable shape, and the exact form of the body may be regarded as a direct adaptation to their mode of life and environment. When living in fluid media, unless the shape is determined by a relatively tough outer membrane or a skeletal support, there is a tendency for the organism to assume the spherical form. Amœbæ, in which an outer membrane is entirely absent or represented by an exceedingly fine pellicle, are spherical unless temporary contractions of the cytoplasm or pressure of any body against which they come in contact or over which they are moving overcomes the physical forces to which they are subject (Fig. 5). So soon as relaxation occurs the spherical form is resumed. In the majority of Protozoa the body is definitely elongated even in a condition of repose, and it is evident that this form is retained without any effort on the part of the organism itself. This is due in most cases to the development of an elastic outer layer of cytoplasm, which retains its shape unless this is temporarily altered by pressure or the contractions of the cytoplasm (Fig. 6). This outer layer of the cytoplasm or periplast may attain a high degree of complexity. It may be so tough, as in many of the Mastigophora and Ciliata, that the shape of the body is practically constant.

In the case of certain Mastigophora, like *Cercomonas*, which are adapted to a creeping mode of existence as well as a swimming one, the body is constantly changing its shape when it is moving over a surface, with a tendency to the assumption of an elongate form during progression through a fluid (Fig. 7). In the majority of Mastigophora and Ciliata which swim through liquids the body is elongated, and may even have a spiral form, when movement is associated with revolution about the longitudinal axis (Figs. 143 and 509). Certain Mastigophora and Ciliata which lead a swimming existence as well as a creeping one upon the surface of various objects are frequently flattened dorso-ventrally. In the swimming forms there is a tendency for one end of the organism to be more pointed than the other. Certain Protozoa become permanently

attached to objects by means of filaments, and in such cases a cone-shape is developed, the filament of attachment arising from the apex of the cone (Fig. 19). Amongst truly parasitic Protozoa the body may be a motionless sphere, as in the growing phases of coccidia within the cytoplasm of cells; on the other hand, those which live in fluids in the body spaces and are endowed with powers of active movement, like free-living forms, vary considerably in shape.

Amongst the Rhizopoda the body is usually either globular or irregular in

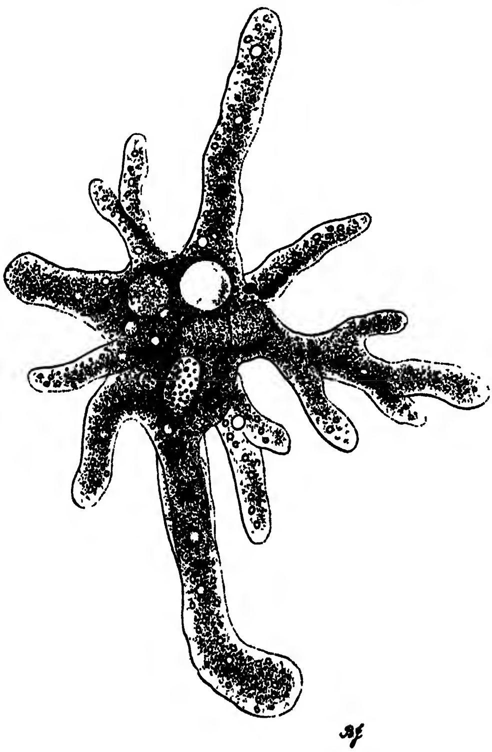


FIG. 5.—*Amœba proteus* (×200). (AFTER LEIDY, 1879.)

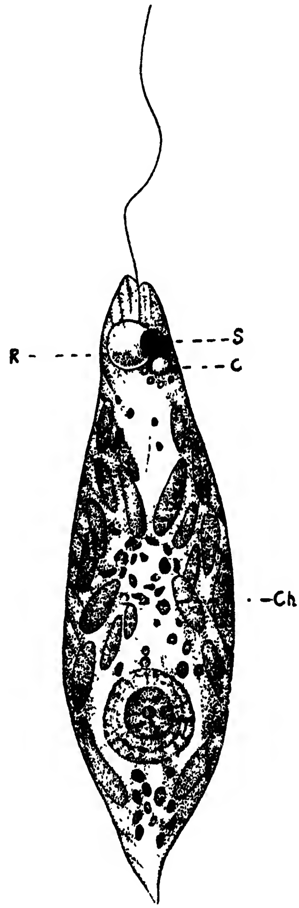


FIG. 6.—*Euglena viridis* (× *ca.* 1,000). (AFTER DOFLEIN, 1916.)

C, Contractile vacuole; Ch, chromatophores; R, reservoir; S, stigma.

shape, and there is no differentiation between an anterior and posterior end or a dorsal or ventral surface (Fig. 5). In certain forms, however, the body is protected by a shell, through an aperture in which pseudopodia are extruded for purposes of locomotion and capture of food. In such forms, of which *Arcella* and *Difflugia* are examples, it is possible to con-

sider the aperture which is applied to the surface over which the organism is moving as ventral in position, so that a dorsal and a ventral surface can be distinguished (Fig. 8). Many Mastigophora are definitely elongate,

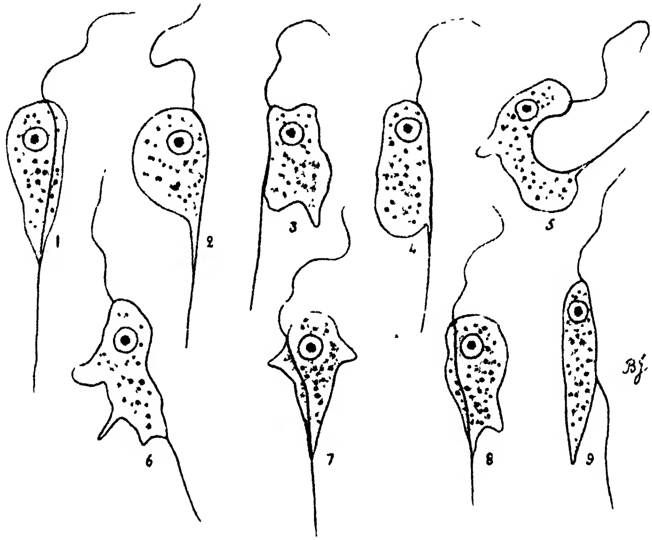


FIG. 7.—*Cercomonas longicauda* (×2000): CHANGES IN SHAPE UNDERGONE BY A SINGLE INDIVIDUAL DURING TWENTY MINUTES' OBSERVATION. (ORIGINAL.)

and locomotion takes place in the direction of the flagellate end (Fig. 6). In these it is evidently possible to distinguish an anterior from a posterior end. When a mouth aperture or cytostome is present, it is usually near the anterior end, but slightly to one side of the terminal flagella. The surface nearest to which the cytostome lies may be regarded as the ventral surface, in which case it becomes possible definitely to orientate the organism. In the case of such a flagellate as *Trichomonas* (Fig. 26) it is legitimate to speak of the anterior flagellated extremity of the body, the posterior extremity through which the axostyle protrudes, the ventral surface near which the cytostome is placed, and the dorsal surface which is provided with the undulating membrane and its basal fibre. This orientation becomes complicated to a certain extent by the fact that a torsion or twisting of the body towards a spiral

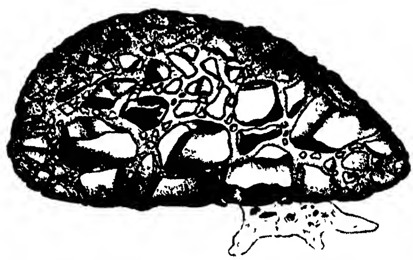


FIG. 8.—*Difflugia constricta*: A SHELLED RHIZOPOD FROM POND WATER (×660). (ORIGINAL.)

The shell is strengthened by adherent grain of sand.

form may occur. Thus in *Trichomonas* itself the undulating membrane takes a slightly spiral course round the body, though its general tendency is to be on the dorsal surface. Amongst the Ciliata this differentiation may be carried to a high degree of complexity. In a few forms such as *Prorodon teres* (Fig. 24) the cytostome is at the extreme anterior end of the body, and the cilia pass in longitudinal rows from it to the posterior end. Though it is possible in these cases to distinguish an anterior and posterior end, there is actually no dorsal or ventral surface. In other forms the cytostome has moved from its terminal position, and it at once becomes possible to regard the surface on which the cytostome is situated as the ventral one

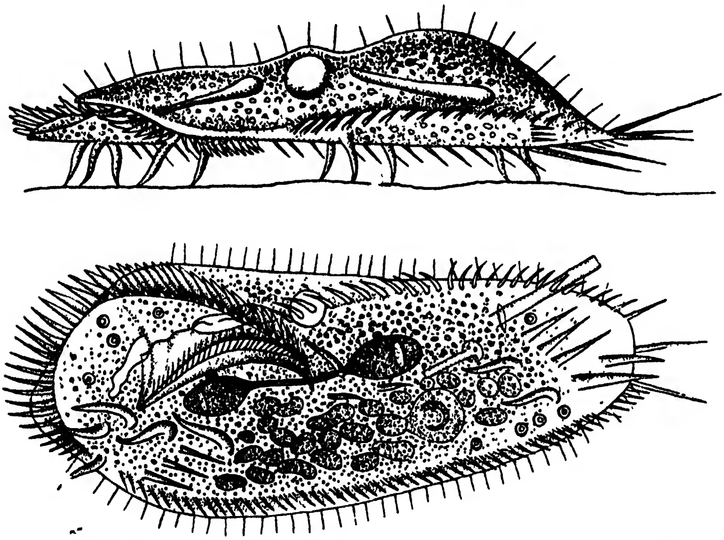


FIG. 9.—*Stylonychia mytilus:* SIDE AND VENTRAL VIEWS (× *ca.* 250). (FROM LANG, 1901, SLIGHTLY MODIFIED.)

The side view shows the ciliate resting on a surface by means of the foot-like cirri formed by fusion of groups of cilia. The dorsal cilia are few in number. There is a central contractile vacuole with two excretory canals leading to it.

The ventral view shows the macronucleus in division and two daughter micronuclei. The V-shaped peristome is bordered on its outer edge by a row of membranes passing round the anterior end of the ciliate and leading to the cytostome at the apex of the V. A row of cilia borders the other edge of the peristome, within which is a longitudinal membrane. The contractile vacuole and parts of the canal are seen as clear areas.

(Fig. 14). In the majority of the free-living Ciliata there are definite dorsal and ventral surfaces. These are most conspicuous in those forms which lead a creeping mode of life, owing to the loss of cilia on the dorsal surface, and the development of cirri and membranelles, through the fusion of groups of cilia, on the ventral surface (Fig. 9). The cytostome is on the ventral surface; it is not median in position, but displaced to one side. In the case of attached forms such as *Vorticella* (Fig. 19)

the ciliated area in which the cytostome lies may be regarded as the ventral surface, and the filament of attachment as a development from the apex of the cone-shaped dorsal surface.

POLYMORPHISM.—It has to be recognized that amongst the Protozoa variations in the shape and form of the body occur at different stages of development. Such a variation is not a characteristic feature of the Rhizopoda, for the smallest individuals of any species have essentially the same body form as the fully-grown larger ones. Amongst the Dimastigamœbidæ at certain stages of development one or more flagella are formed. Though it is purely an arbitrary matter whether the flagellate stage is considered to be the adult form or not, these amœbæ are definitely polymorphic (Figs. 119 and 120). As all Rhizopoda, however, are able to encyst under certain conditions, the encysted stage has to be recognized as another form in which any particular amœba may occur. Amongst the free-swimming Ciliata, again, the smallest individuals differ little except in size from the fully-grown largest forms.

Protozoa which only show a slight degree of variation in body form during their life-cycle are termed *monomorphic*, to distinguish them from *polymorphic* forms, which show this to a marked degree. This polymorphism is well illustrated by the development of the Suctoria (Fig. 532). Amongst these Protozoa the attached adult buds off a small ciliated embryo, which, after leading a free-swimming existence for a time, finally attaches itself, loses its cilia, and grows into the adult, which is provided with sucking tentacles. As the ciliated stage is only of a temporary nature, and is small when compared with the tentacled stage, it is regarded as the embryo. Amongst the Sporozoa there is a high degree of polymorphism associated with their complicated cycles of development. In the case of the malarial parasites, for instance, the organism passes through a constant series of changes of form (Fig. 391). The minute amœboid organism within the red cell grows into the schizont, which breaks up into elongate merozoites, which again become amœboid forms in other cells. Some merozoites develop into gametocytes of two types, which can be distinguished from the schizonts. In the mosquito the gametocytes change in character and produce elongate vermicular zygotes, which pass through the stomach wall and develop into oöcysts, which again produce a large number of minute sickle-shaped sporozoites, which differ in character from the merozoites. In this case, as in other Sporozoa, there is a high degree of polymorphism, as exhibited by a constant series of changes in the size and form of the body. Very frequently there is a polymorphism associated with the occurrence of a sexual process and the formation of gametes. In the gregarines the gametes which unite may be exactly alike, in which case the process is known as one of *isogamy*. On the other hand, in certain

gregarines the uniting cells differ from one another (*anisogamy*), so that there is a degree of polymorphism as regards the character of the gametes (Fig. 482). It may happen that the individual which gives rise to gametes of one type differs from that which gives rise to gametes of the other type. This differentiation may extend further back in the life-history, so that it is possible to recognize two distinct types of reproducing individual, each with its particular characters (Fig. 341). The individuals of one series may eventually, after a period of multiplication, give rise to gametes of one type, while those of the other series give rise to gametes of another type. In such cases it might be supposed that one was dealing with two distinct organisms, each reproducing its kind. The fact that the gametes produced by the one unite with those produced by the other proves that the two series belong to one polymorphic species. This condition is known as one of *sexual dimorphism*, a term which is also employed in a more general sense to indicate the occurrence of individuals of any species which can be distinguished as male and female.

Though all these variations in form, which occur as a result of growth, complicated life-cycles or the sexual process, are examples of polymorphism, the term is often employed in a more restricted sense. When it has been decided which stage of the organism is to be regarded as the adult form, it may be found that the adults resemble one another very closely, in which case the organism is said to be monomorphic. Thus, in the case of trypanosomes the commonly observed forms in the blood of an animal may vary very slightly. In these cases the organism is termed a monomorphic trypanosome, examples of which are *Trypanosoma evansi* and *T. congolense* (Figs. 227 and 234). In other cases, as, for instance, *Trypanosoma brucei*, it may be possible to distinguish in the blood of an animal several distinct types—long thin, intermediate, and stumpy trypanosomes —and forms with or without free flagella (Fig. 225). On this account *T. brucei* is regarded as a polymorphic trypanosome. If, however, the whole life-cycle in the vertebrate and invertebrate hosts of such a form as *T. lewisi*, which at certain phases appears monomorphic, is taken into consideration, it will be found to exhibit as great a degree of variation as in the polymorphic trypanosomes (Fig. 197).

It seems clear, therefore, that the term "polymorphism" is incapable of exact definition. Strictly speaking, no Protozoan is monomorphic, while all are polymorphic. Those which are considered monomorphic show only a slight degree of variation, while those which are polymorphic show the variations, but to a greater extent. Any organism may be regarded as polymorphic because it differs at different stages of its growth and life-history, or it may be considered as polymorphic because the individuals which have all reached any particular stage do not resemble one another

very closely. Human beings may be regarded as polymorphic because the child differs from the adult, or they may be considered polymorphic because the adults differ amongst themselves. It is in the latter sense that the term is commonly employed in connection with trypanosomes. It must be recognized, however, that the trypanosomes which are regarded as being polymorphic may not all be in the same stage of development. There is evidence which points to the fact that the shorter stumpy forms of *T. brucei* or *T. gambiense* are the result of growth from the long slender forms which are present in the blood at the same time (Figs. 222 and 225).

RACES.—Amongst Protozoa, as amongst human beings, there occur different races of one and the same species. The individuals of one race differ from those of another in size, shape, rate of multiplication, and other characters. Each race breeds true to its type to a large extent, so that even after long periods of multiplication the same differences are observed in the resulting progeny. On this account it often becomes a matter of difficulty to decide whether two different forms are merely races of one species or are actually different species. Thus, in the case of *Entamœba histolytica* there appear to be several races which can be distinguished from one another by the average size of the cysts they produce (Fig. 10).

Many researches have been conducted on the race question in species of *Paramecium*, *Difflugia*, and other Protozoa, especially by Jennings. It has been observed that the characters of any particular race tend to remain constant, so that there is considerable difficulty in understanding how these races arose in the first instance. Evidence has, however, been obtained by Jennings (1916) in the case of *Difflugia corona* and by Middleton (1915) for *Stylonychia*, which proves that after long periods of multiplication definite inheritable variations do occur in the descendants of a single individual, and this quite apart from any sexual process. It therefore seems probable that if the observations were continued for a sufficient length of time, it would be possible to separate from the descendants of a single individual various races which would be as distinct from one another as the naturally occurring races. If this were not so, it would be difficult to understand how evolution could take place at all.

A practical point which arises from the knowledge which has been acquired regarding races of Protozoa is that the separation of species, on account of comparatively slight variations in size, is a very questionable procedure. The literature dealing with parasitic Protozoa contains numerous instances of the establishment of new species merely because the dimensions differed slightly from those of a form previously described.

Another type of race peculiarity occurs amongst the Ciliata. It was shown by Dawson (1919) that *Oxytricha hymenostoma*, which normally has both a macronucleus and a micronucleus, may occasionally have the

macronucleus alone. Such an amicronucleate race was cultivated by him for several years, during which regular multiplication by fission took place. Abortive attempts at conjugation appeared to be made, but the process was never completed. Landis (1920) has studied a similar race of *Paramecium caudatum*, and Patten (1921) one of *Didinium nasutum*, while Woodruff (1921*a*) has described amicronucleate races of *Oxytricha fallax* and *Urostyla grandis*.

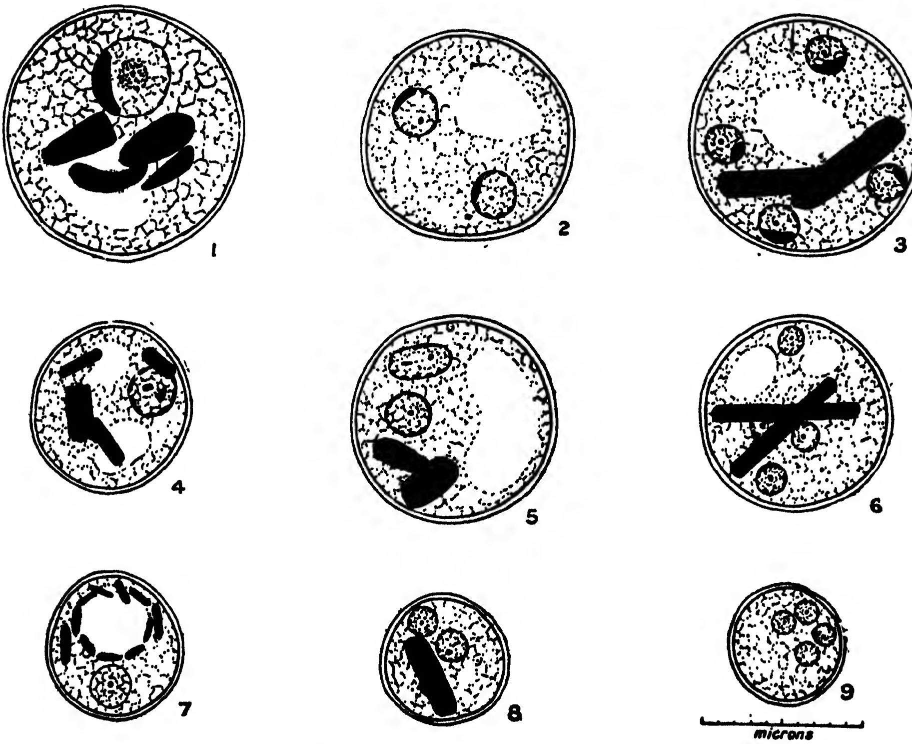


FIG. 10.—CYSTS OF *E. histolytica* FROM THREE DISTINCT RACES (× 2,200). (AFTER WENYON AND O'CONNOR, 1917.)

1-3. Race with exceptionally large cysts. 4-6. Race with usual type of cyst. 7-9. Race with small cysts.

CYTOPLASM.—The cytoplasm of the Protozoan cell does not differ in any essential respect from that of cells of multicellular animals. As to the nature of its minute structure many theories have been advanced. That which seems to be most satisfactory is Bütschli's view that cytoplasm is of the nature of an emulsion consisting of at least two substances, one of which in the form of minute globules is suspended in the other, which forms the septa between the globules. In optical section, the

substance between the globules has the appearance of a network of fibres. Embedded in these apparent fibres or septa are granules of various kinds and sizes. The cytoplasm commonly contains vacuoles, which are spherical spaces containing a material which is more fluid than the constituents of the cytoplasm itself. Very frequently within the vacuoles are food particles which the organism has ingested. In such cases the vacuoles are known as *food vacuoles* or digestive vacuoles, and into them are secreted acid ferments capable of transforming the food into substances suitable for assimilation by the cytoplasm. The products of digestion are gradually absorbed into the cytoplasm, and any residue is got rid of by the vacuole approaching the surface of the body and discharging its contents into the medium in which the organism is living. The vacuole is then no longer visible. In the majority of free-living Protozoa there are one or more vacuoles, which are known as *contractile vacuoles* or pulsating vacuoles. Such a vacuole is near the surface of the body, and when fully formed contains a clear fluid. By a sudden contraction the contents of the vacuole are discharged through the surface of the body, and the vacuole disappears. Very soon, however, a minute vacuole reappears at the same spot. It gradually increases in size owing to the flow of liquid into it, sometimes along definite channels. When it has attained its full size, expulsion of the contents again takes place. These vacuoles appear to be of an excretory nature, and the intervals between the contractions vary with the temperature and other conditions. For some reason not clearly understood, contractile vacuoles are frequently absent in parasitic Protozoa.

Within the cytoplasm of many Protozoa there occur various structures which are to be regarded as secretions of a skeletal nature. In the Heliozoa, for instance, radially arranged rod-like supports for the pseudopodia are formed (Fig. 75), while in many of the Radiolaria complicated fenestrated shells of a spherical or other shape are secreted in the cytoplasm (Fig. 78). These internal structures are not to be regarded as part of the cytoplasm itself, but bear the same relation to it as the external shells and coverings, which are sometimes formed around the organism for protective purposes of a permanent or temporary nature.

A very noticeable feature of the cytoplasm of Protozoa is its differentiation into an *ectoplasm* and an *endoplasm*. The former is of tougher consistency and more hyaline than the endoplasm, and forms a superficial layer of varying thickness enclosing the more liquid and granular endoplasm. The endoplasm, even when the organism is at rest, appears to be constantly streaming in various directions. The different vacuoles and bodies, and even the nucleus itself, are constantly changing position as a result of the currents in the endoplasm. It is in the endoplasm that the various vacuoles and internal skeletal structures occur, while the ectoplasm

may become highly differentiated. A tough covering to the body, which may be elaborately marked, is often developed from the ectoplasm, while it is from this layer that the various permanent organs of locomotion such as flagella and cilia originate. The ectoplasm also secretes the various external coverings, such as shells and cysts. In the simpler Protozoa, like the amœbæ and flagellates, the ectoplasm is merely a thin layer of clear cytoplasm surrounding the endoplasm. It appears to be only slightly more resistant than the endoplasm. In the more highly organized ciliates and gregarines the ectoplasm is highly developed, and itself consists of several distinct layers. It is a resistant membrane which enables the organism to retain its shape. In any case, the most superficial layer of the ectoplasm forms a delicate limiting membrane, the periplast. The surface of the ectoplasm may be perfectly smooth, or it may be raised into a series of longitudinal ridges. In other cases it is roughened, or may even develop a series of symmetrical markings. In the amœbæ, many of the simpler flagellates, and many parasitic protozoa, the ectoplasm forms a complete layer over the surface of the body, and when solid food is ingested this is taken in at any part of the body. A particle comes in contact with the ectoplasm which is gradually raised up round it, and finally closes over it, so that the object, together with a certain quantity of liquid, is included in a vacuole which sinks into the endoplasm. In other cases the solid food particles are ingested in a similar manner at one particular spot on the body surface. This occurs typically in certain flagellates, where solid food appears to be ingested only at the base of the flagellum. In other flagellates at this point there is a small excavation or pit in the ectoplasm into which solid food is taken (Figs. 26 and 33). At the bottom of this pit the food particle sinks into the endoplasm, and is included in a vacuole. This depression is frequently of a permanent nature. In association with it there may be special developments of the organs of locomotion which create currents in the medium, so that food particles are directed into it. In *Chilomastix* one of the flagella lies in a groove, at the posterior end of which food particles enter the cytoplasm (Fig. 69). The opening in the ectoplasm, which sometimes is capable of being opened and closed, is known as the *cytostome*, while the funnel-shaped pit or tube leading from it to the endoplasm is the œsophagus or *cytopharynx*.

As already pointed out, the residue from the digestion of food material within the food vacuoles is discharged through the surface of the body. This may occur at any point on the body surface, but in the Ciliata there may be a permanent opening in the ectoplasm, the *cytopyge*, which, however, is usually only visible when a food vacuole discharges its contents at the posterior end of the body (Fig. 512).

In some ciliates the cytostome is a simple opening on the surface of the

body, but the region round the cytostome (*peristome*) may be modified in various ways. There may be a ciliated groove leading to the cytostome (Fig. 70), or a disc-like area upon which cilia are arranged in a spiral manner (*adoral zone of cilia*) may be developed. These cilia are often continuous with others within the cytopharynx. In the Peritrichida, like *Vorticella* and *Carchesium*, the area round the cytostome is sunk in the form of a funnel-shaped depression, the *vestibulum*, the opening of which may be completely closed by contractions of the cytoplasm. Within the vestibulum is found the cytostome itself, while the food vacuoles and contractile vacuole also discharge their contents into it (Fig. 528).

CYTOPLASMIC INCLUSIONS.—In association with the ingestion of food and metabolism, granules, globules, and crystals of various kinds may appear in the endoplasm. These are quite distinct from the partially digested food in the food vacuoles, though they result from food metabolism. Many Protozoa having affinities with the plants and possessing chlorophyll are able to form starch, which occurs in the cytoplasm as characteristic starch granules. They are commonly present in *Euglena* and other similar forms. Another substance allied to starch is known as paramylum. Fat globules are seen especially amongst the Radiolaria within the inner capsule. They also occur in the marine flagellate *Noctiluca*, and it has been suggested that they assist these organisms to float. Doflein (1910) has noted that, in old cultures of *Trypanosoma rotatorium* the flagellates may contain droplets of fat. Another substance which is of common occurrence in the cytoplasm is glycogen, or a closely allied substance which was called paraglycogen by Bütschli. These have a strong affinity for iodine, which colours them an intense brown. Glycogen is present in gregarines, certain ciliates, and very commonly in the encysted forms of amœbæ and flagellates (Plate II., p. 250). The iodophilic body which occurs in the encysted stage of *Iodamœba bütschlii* has given rise to its generic name. A substance which is of wide distribution amongst the Protozoa is volutin. It is usually seen in living organisms as globules of a greenish refractile material which takes a yellow colour in iodine. Owing to the fact that it may stain deeply with chromatin stains, it has often been regarded as chromatin. Some observers maintain that it is actually a forerunner of chromatin. Volutin is often present in the cytoplasm of trypanosomes and other flagellates, and appears as dark red granules when they are stained with Romanowsky stains. It commonly occurs in hæmogregarines and many Sporozoa, as also in amœbæ and ciliates. A substance which may be allied to volutin is seen in the chromatoid bodies which are present in the cysts of some intestinal amœbæ. They occur so frequently in the encysted forms of *Entamœba histolytica* in the form of bars that they are highly characteristic of this species

(Fig. 96). They are less often seen in the encysted stages of *Entamœba coli*. Like the glycogenic or iodophilic body in the encysted form of *Iodamœba bütschlii*, they disappear in the course of a few weeks after escape of the cysts from the intestine, apparently serving as a supply of nourishment for the enclosed amœbæ.

Another type of cytoplasmic inclusion is the chromatophore, which is characteristic of many plant-like flagellates grouped amongst the Phytomastigina (Fig. 130). These are bodies which contain various pigments known as chromatophyll. When green it is called chlorophyll, and when red hæmatochrome. As in plants, these bodies enable the organism to utilize the carbonic acid of the medium in which they live. It has been shown that the chromatophores multiply by fission in the cytoplasm, as also do certain granules known as pyrenoids which may be present in the chromatophores. It has been surmised that the chromatophores may be symbiotic organisms living in the cytoplasm.

In the process of ingesting solid food many Protozoa actually ingest other forms, or even their own species, either in the free or encysted condition (Fig. 99). The writer has seen a large vacuole in *Entamœba muris* of the mouse filled with actively motile *Trichomonas*. The intestinal amœbæ of man frequently ingest the encysted forms of other intestinal Protozoa. In many cases the ingested organisms are killed and digested, but this is not always the case. Instances are known in which amœbæ and even ciliates may have their cytoplasm riddled with vacuoles in which smaller amœbæ or flagellates occur. These may eventually escape apparently unharmed by their stay in the cytoplasm of another organism. Protozoa are also liable to invasion by bacteria. Such a condition approaches, and may actually be, one of parasitism. Instances of true parasitism are seen in the invasion of the body of *Paramecium* by the Suctorian *Sphærophrya pusilla* (Fig. 534), and of various intestinal flagellates and amœbæ by *Sphærita*, a vegetable organism which often resembles a cluster of large cocci (Fig. 111, 4). The inclusion of smaller organisms within the cytoplasm of larger ones has always to be remembered, especially when a process of multiplication by the production of daughter individuals within the cytoplasm of a parent is considered. The nuclei of such forms may be mistaken for nuclei belonging to the host. A method of reproduction of *Pelomyxa palustris*, a large multinucleated amœba, by the production of flagellated forms within vacuoles in its own cytoplasm has been described by Schirch (1914). It seems not improbable that this was an instance in which an amœba had ingested, but failed to kill, a number of flagellates which were present in the medium. Doflein (1916) mentions an instance in which the cytoplasm of a ciliate, *Stentor cæruleus*, included numerous small flagellate organisms.

ORGANS EMPLOYED IN LOCOMOTION AND CAPTURE OF FOOD.—The simplest organs which are used for purposes of locomotion are the *pseudopodia*, characteristic of the Rhizopoda or amœbæ (Fig. 5). They are simply processes of cytoplasm which are formed from the surface of the body. A small elevation of the ectoplasm occurs at any point, and this gradually increases in size till the endoplasm also takes part in its formation. When it has reached a certain size it may be withdrawn gradually, and another formed in some other direction. On the other hand, it may increase steadily in size till the whole body of the organism flows into it. In this manner, by the regular production of pseudopodia, an amœba may move from one spot to another. It is by means of pseudopodia passed around any object that food particles are ingested. The movements and changes in shape associated with the formation and withdrawal of pseudopodia are termed *amœboid movements*, which are exhibited typically by the amœbæ. Certain flagellates as well as Sporozoa, such as the malarial parasites, may also move in this manner. The pseudopodia may be blunt finger-like processes of a *lobose* type, or they may be relatively long, thin, and tapering, and of a *filose* type. The long narrow filose pseudopodia may remain separate from one another, or they may become united by lateral anastomoses, so that an organism possessing many of them appears to be surrounded by a fine network of cytoplasm, as in the Foraminifera (Fig. 72). In the case of the Heliozoa and Radiolaria, the filose pseudopodia are more permanent structures, known as *axopodia*, and are supported by radially arranged axial rods secreted by the endoplasm, or formed as outgrowths from the central granule (Fig. 51).

Flagella and cilia are more permanent organs of locomotion. The former are characteristic of the Mastigophora, and the latter of the Ciliophora. They are long, narrow, whip-like processes which are capable of performing undulating or lashing movements, which cause currents in the medium and enable the organism to progress through it. A single flagellum has essentially the same structure as a cilium, though it is usually larger, and is capable of more violent lashing movements. Generally speaking, the small number of flagella possessed by a flagellate fulfils the functions of the large number of cilia possessed by a ciliate. A flagellum, as pointed out by Alexeieff (1911*e*), consists of an axial filament, for which the term *axoneme*, suggested to the writer by Colonel A. Alcock, will be employed, and a thin sheath of cytoplasm (Fig. 157). The axoneme itself takes origin in a minute granule, the *blepharoplast*, which is situated in the cytoplasm, and sometimes upon the surface of the nuclear membrane. The axoneme passes to the surface of the body, and there, acquiring a thin sheath of cytoplasm, becomes the flagellum. There can thus be distinguished an intracytoplasmic portion of the axoneme and a flagellar

portion. For the former the name *rhizoplast* is often employed. When an organism is developing a flagellum, a blepharoplast first becomes apparent in the cytoplasm, and an axoneme is formed as an outgrowth from it. When the surface of the body is reached, increase in length still takes place, the axoneme pushing out a thin covering of cytoplasm. It is probable that the axial rod of an axopodium is a homologue of the axoneme of a flagellum.

The flagella of the Mastigophora vary in number. In the typical forms they are not numerous. There may be only a single one, or as many as eight. They arise most usually from the anterior end of the body, and are directed forwards. By their lashing movements they propel the organism through the medium. In some instances certain flagella arise from the posterior end of the body, and are directed backwards. Thus, in *Hexamita* two of the eight flagella are posterior in position, but their axonemes can be traced through the cytoplasm to the anteriorly situated blepharoplasts (Fig. 288). In other cases, as in *Tricercomonas* and *Cercomonas*, the axonemes of the posterior flagellum can be traced over the surface of the body to the anterior end, where it enters the cytoplasm and passes to the blepharoplast (Figs. 259 and 261). In the flagellates of the genera *Trypanoplasma* and *Trichomonas* such a backwardly directed axoneme is adherent to, or embedded in, the margin of a thin band of cytoplasm, the undulating membrane (Figs. 26 and 151). In other cases, such as *Bodo*, one flagellum is directed backwards, and acts as a trailing flagellum without being attached to the surface of the body (Figs. 21 and 33). In the case of the trypanosomes, the blepharoplast occupies an unusual position at the posterior end of the body. The axoneme arising from it is directed forwards, and passes over the surface of the body or along the margin of an undulating membrane as far as the anterior end of the body, where it either terminates or becomes a flagellum (Fig. 28, B).

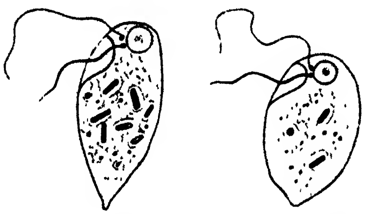


FIG. 11.—*Embadomonas* SP. FROM CULTURE OF INTESTINAL CONTENTS OF *Testudo argentina* (× *ca.* 1,500). (ORIGINAL.)

All the flagella possessed by a flagellate may be uniform as regards length and thickness when they fulfil the same function. Frequently, however, variations occur. In the case of *Embadomonas*, one of the two flagella, which organisms of this genus possess, is associated with the cytostome, and is much thicker and shorter, and performs more regular undulating movements than the anteriorly directed one (Fig. 11). Flagella are employed not only for purposes of progression, but also for

the capture of food. A cytostome, when present, is always near the point of origin of the flagella, one of which may be specially modified in connection with the cytostome. Thus, in *Chilomastix* one flagellum is permanently within the cytostomal groove, where it functions by creating currents which assist in the capture of food (Fig. 69). The thicker of the two flagella possessed by *Embadomonas* has a similar function (Fig. 11).

As already remarked, in typical flagellates the flagella are few in number, but there occur certain forms which possess many flagella.

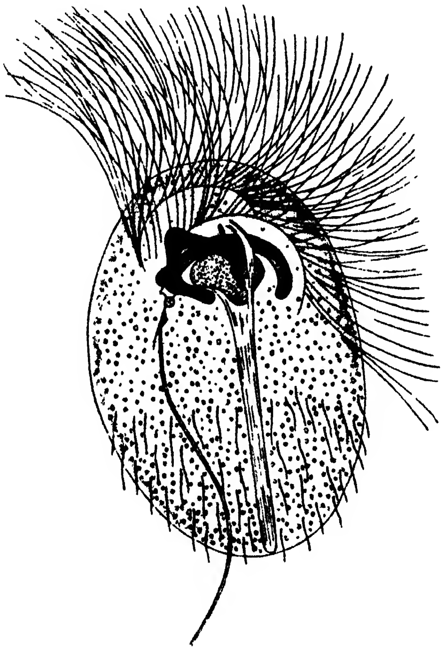


FIG. 12.—*Parajœnia grassii* (× 1,500). (AFTER JANICKI, 1915.)

FIG. 13.—*Holomastigoides hertwigi* (× 320). (AFTER HARTMANN, 1910.)

These are the Hypermastigida, which occur chiefly as intestinal parasites of white ants (Figs. 12 and 13). They stand in this respect as a connecting link between the Mastigophora and the Ciliophora, with both of which groups observers have classed them. Though the possession of flagella is a characteristic feature of the Mastigophora, it must be remembered that these organs of locomotion are not peculiar to this group. Certain forms which are classed with the Rhizopoda, and which are amœboid organisms, may have flagella at certain stages of development. Similarly, amongst the Sporozoa the microgametes are commonly supplied with one or two

flagella, which enable them to move about in search of the macrogametes (Fig. 337).

As noted above, the cilia which characterize the Ciliophora resemble small flagella. They have a similar structure, and their axial fibres take origin in minute granules situated in the ectoplasm. It seems reasonable to suppose that the axial fibres and the basal granules of cilia are homologous with the axonemes and blepharoplasts of flagella. A single ciliate possesses a large number of cilia, which exhibit more regularity and co-ordination in their movements than the flagella of one of the Mastigophora. In some ciliates the body is covered uniformly with cilia, which, however, are usually arranged in longitudinal rows (Fig. 14). In other cases the cilia are limited to special regions of the body. The cilia may be fairly uniform in length, but frequently those on the extremities of the body and those which surround the cytostome are slightly longer than the others. Cilia are often continued into the cytopharynx. Sometimes, as in *Cyclidium* and other forms, one posterior cilium is much larger than the others, and forms a kind of tail or caudal process which has very much the same size and structure as a flagellum (Fig. 500). Several adjacent cilia may fuse together to form stout processes known as cirri. These are seen typically on the ventral surface of those ciliates (Hypotrichida) which lead a creeping mode of existence (Fig. 9). They function as supporting structures or legs. In some cases, again, rows of cilia may unite to form membranes. This occurs frequently in the cytopharynx of certain ciliates, such as *Paramecium*, *Pleuronema*, and others (Fig. 70). These membranes, or membranelles as the small ones are often named, are distinct from the undulating membranes of Mastigophora (trypanosomes), which are thin ridges of ectoplasm, and are not formed by the fusion of rows of cilia. The cilia on the peristome region near the cytostome may differ little from those on other parts of the body. On the other hand, they may be considerably modified in character and arrangement. In many forms they are arranged as a spiral to form the adoral zone of cilia, which are continuous with those in the cytopharynx. The spiral may be a left-handed spiral or a right-handed one. It may consist of only a single turn or part of one.

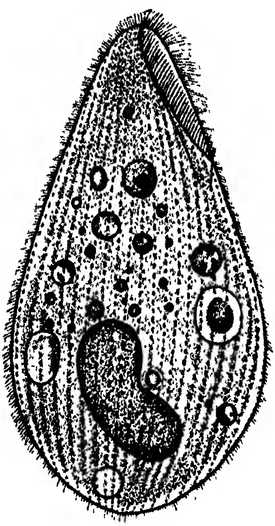


FIG. 14.—*Balantidium entozoon* FROM THE RECTUM OF THE FROG (×650). (ORIGINAL.)

The longitudinal rows of cilia on the surface of the body are represented by dots.

or there may be as many as five complete turns. The spiral may be compared with a portion of a flat watch-spring, the cytostome being situated at the outer end of the spiral, which lies on the peristome area in front of the cytostome. The cilia composing the spiral generally consist of several

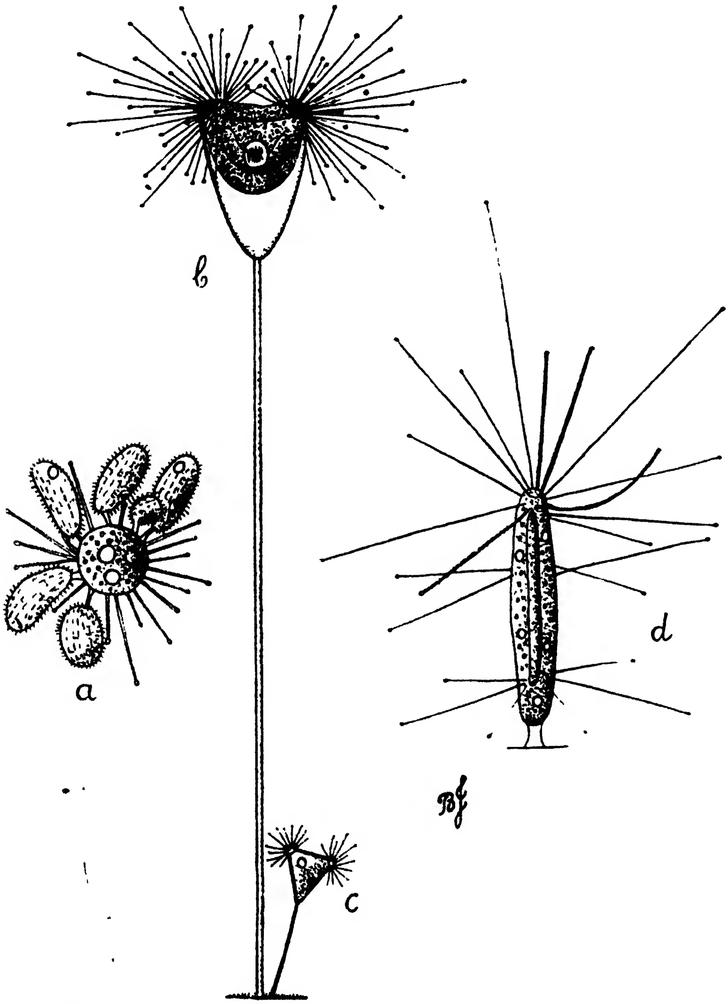


FIG. 15.—VARIOUS SPECIES OF SUCTORIA. (AFTER SAVILLE KENT, 1880-1882.)

(*a*) *Sphærophrya magna* feeding on ciliates (× 300). (*b*) *Acineta grandis* (× 100).
(*c*) *Tokophrya lemnarum* (× 100). (*d*) *Discophrya elongata* (× 150).

parallel rows, and those of adjacent rows may unite in such a way as to form a series of spirally arranged, flat, tongue-like processes or membranelles (Fig. 511). Within the cytostome the cilia may fuse to form one or more membranes parallel to the axis of the cytopharynx. The general

structure and arrangement of the cilia on the body of ciliates and the modifications undergone by those associated with the cytostome are features of importance in the classification and determination of the species and genera of the Ciliata, just as the number and characters of the flagella are of importance in the classification of the Mastigophora.

Amongst the Suctoria, which in their youngest stages are provided with cilia, special organs for use in nutrition are developed in the adults

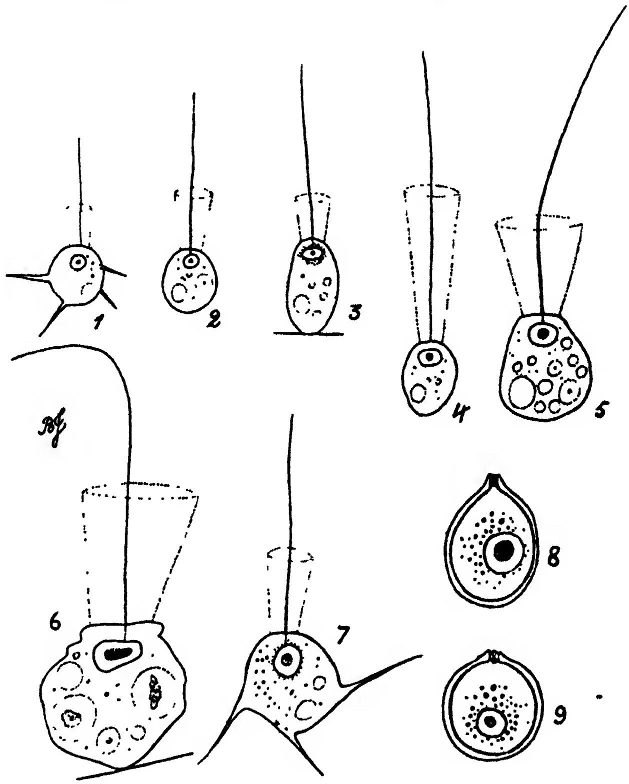


FIG. 16.—*Monosiga consociatum* FROM HAY INFUSION (× 2,000). (ORIGINAL.)
1-7. Free and attached individuals of varying size. 8 and 9. Encysted forms.

(Fig. 15). These are known as tentacles, and each is a tubular process of cytoplasm terminating in a disc-like sucker. The latter is applied to food material, which is taken into the body by a sucking process. It is the possession of these sucking tentacles which has given rise to the names Suctoria and Tentaculifera, by which these forms are known.

Another type of structure which probably has to do with the capture of food is the thin collar which is developed in certain Mastigophora (Fig. 16). The cytoplasm at the anterior region of the body is raised

into a thin cylindrical collar or cuff round the flagellum. The collared forms frequently possess attachment filaments, simple or branched, and often cup-like loricæ. The collared forms are generally known as the Choanoflagellata. Similar flagellated collar cells are found in the group of Metazoa to which the sponges belong. In many cases it appears that the collar is not a cylinder, but a cuff with overlapping edges.

A peculiar modification of the ectoplasm which facilitates locomotion occurs in gregarines. These organisms are able to glide over a surface without exhibiting any movements of contraction of the body by reason of longitudinal ridges of ectoplasm between which a quantity of mucoid material can be rapidly excreted. The excretion of this tenacious material

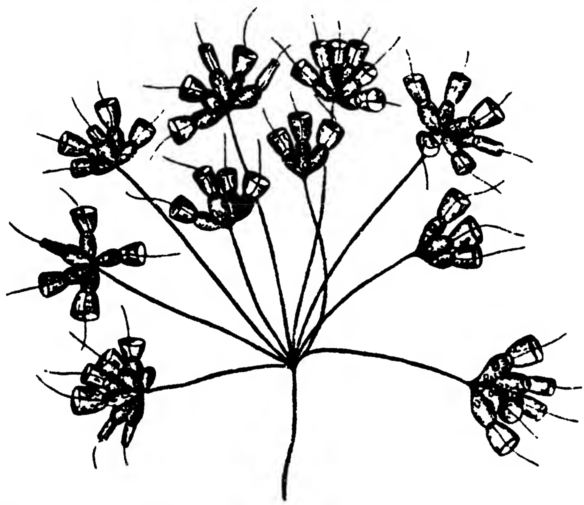


FIG. 17.—*Codonosiga allioides:* A COLONY OF COLLARED FLAGELLATES ON A BRANCHED FILAMENT (×320). (FROM LANG, 1901, AFTER KENT.)

causes the organism to be pushed forwards without any apparent movements of the body. Similar gliding movements are often exhibited by the merozoites or sporozoites of the Sporozoa. In the case of certain amœbæ such a gliding movement appears to be the result of constant streaming of the cytoplasm from behind forwards, while the ectoplasm in contact with the surface remains stationary, very much as a bag of water can be pushed along the surface of a table.

ORGANS OF ATTACHMENT.—Though the majority of the Protozoa are free-living organisms, certain forms are able to attach themselves temporarily or permanently to objects.

Amongst the Mastigophora there are many pedunculated forms. The posterior extremity of the body is developed into a filament, by means

of which fixation to various objects is brought about (Fig. 18). Such forms are more or less permanently attached. By longitudinal division of the attached flagellate and the continued development of the filament

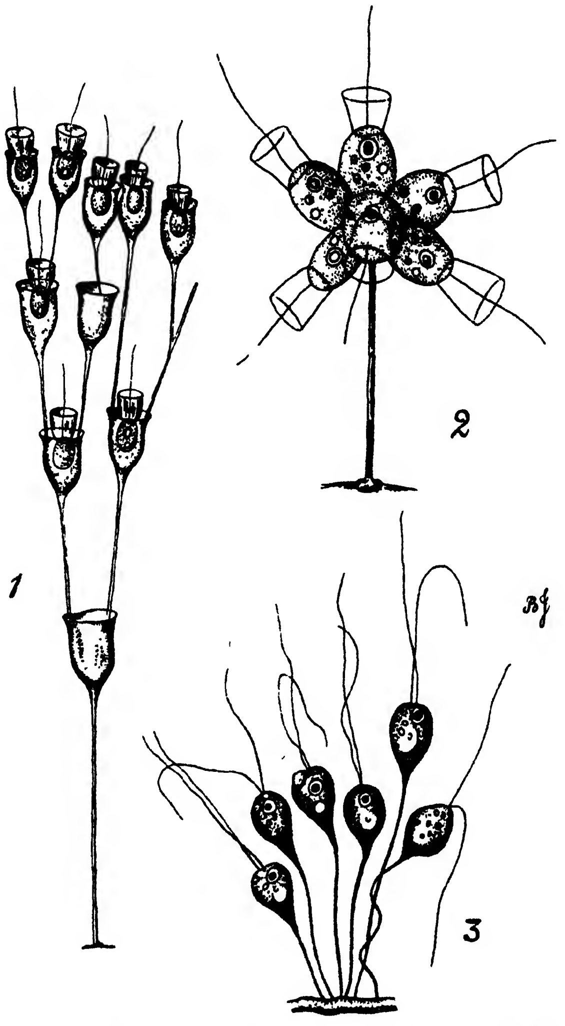


FIG. 18.—VARIOUS ATTACHED FLAGELLATES. (1, FROM LANG, 1901, AFTER KENT; 2, FROM LEMMERMANN, 1914, AFTER KENT; 3, AFTER DOFLEIN, 1916.)

1. *Poliœca dichotoma* (× 1,000). 2. *Codonosiga botrytis* (× 1,200). 3. *Amphimonas globosa* (× 1,500).

from the posterior end of the body complicated branched filaments are developed (Fig. 17). Sometimes the end of the branch is continued round the organism as a cup-like expansion or lorica, in which it lives

(Fig. 18, 1). Similarly, amongst the Ciliata filaments of attachment, either simple or branched, may be developed. In some cases, as, for example, *Vorticella*, the filament contains a contractile thread, by means of which it can be suddenly coiled up in a spiral manner and the ciliate withdrawn when it is subject to adverse stimuli (Fig. 19).

Amongst parasitic Protozoa, many gregarines are provided with special organs of attachment. The young organism which develops from the sporozoite is at first intracellular, but as growth occurs it leaves the host cell, to which, however, it remains attached by a process known as the *epimerite* (Fig. 20). This structure is developed in various ways, and may be compared to the organ of attachment of tape-worms. It

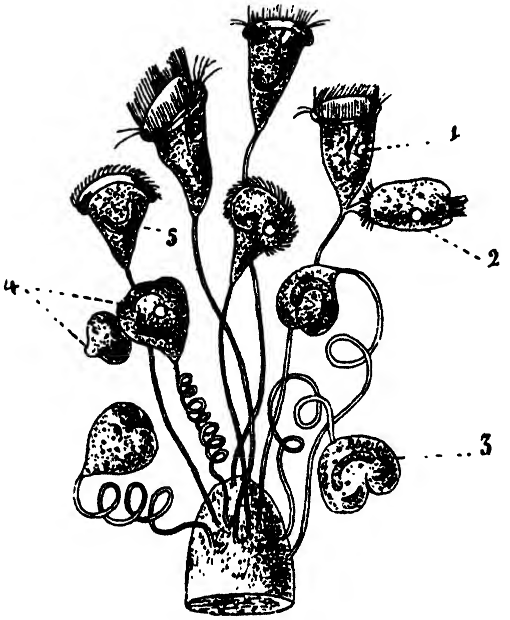


FIG. 19.—*Vorticella nebulifera*: A GROUP OF STALKED CILIATES ATTACHED TO AN OBJECT (× 200). (FROM LANG, 1901, AFTER D'UDEKEM.)

1, Contractile vacuole; 2, daughter individual with circlet of cilia; 3, dividing form; 4, conjugation.

FIG. 20.—A CEPHALINE GREGARINE (*Corycella armata*) (× *ca.* 300), SHOWING EPIMERITE, PROTOMERITE, AND DEUTOMERITE. (AFTER LEGER, 1892.)

may be a simple swollen body embedded in the cytoplasm of the cell, and connected with the parasite by a kind of neck, or there may be developed from it a series of filaments or roots which anchor the parasite to the cell. In some cases a large sucker-like process is applied to the surface of cells, and from it a series of filaments pass into the cells or between adjacent cells. In other cases the epimerite is supplied with a

series of small spines. After growth of the gregarine is complete, the epimerite is detached (Figs. 481 and 485).

Many Mastigophora are able to attach themselves temporarily to objects. This is generally effected by a flagellum, as in species of *Bodo* (Fig. 21), but some forms, like *Oikomonas*, can become fixed by a pseudopodium-like process developed from the posterior end of the body. In the case of trypanosomes and their allies attachment to cells is an important feature of development in the invertebrate host. In the intestine, proboscis, or salivary gland of insects in which development is taking place, large numbers of the flagellates may be attached to the surface of the cells, and as longitudinal division may take place while they are attached, the surface of the cells may become completely covered with attached organisms. In this process, what usually happens is that the flagellum disappears, attachment being effected by the tip of the axoneme (Fig. 150).

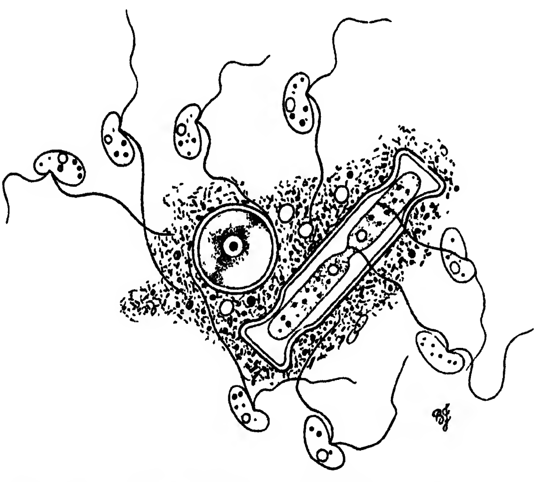


FIG. 21.—*Bodo saltans:* A NUMBER OF INDIVIDUALS ATTACHED TO A MASS OF DEBRIS BY THE TRAILING FLAGELLA (× 1,000). (ORIGINAL.)

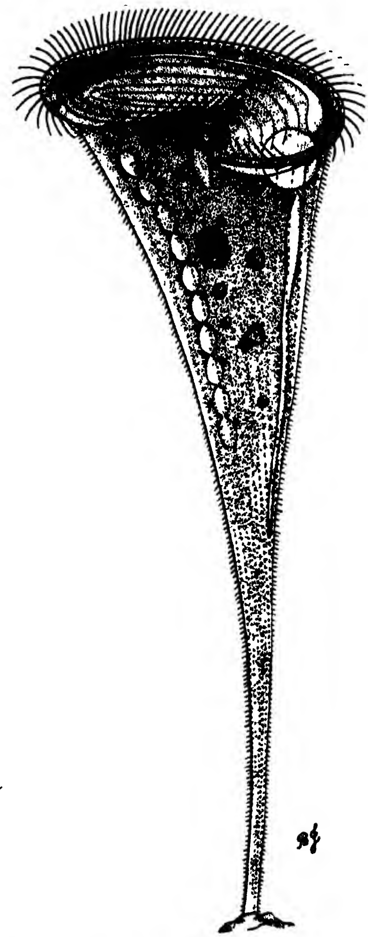


FIG. 22.—*Stentor cœruleus* (× 146). (ORIGINAL DRAWING FROM LIFE BY B. JOBLING.)

In some Protozoa there is a sucker-like development of the surface

of the body which enables the organism to attach itself temporarily. In the case of *Giardia* (*Lamblia*) the ventral surface develops a large sucking disc, by means of which the flagellate is able to attach itself to the surface of the intestinal cells (Fig. 291). Amongst the Ciliata *Stentor*, which is conical in shape, is able to fix itself to objects by pseudopodium-like processes at its tapering posterior end (Fig. 22).

SKELETAL OR SUPPORTING STRUCTURES.—It has already been pointed out that some Protozoa are able to build for themselves protective external coverings. Amongst the Rhizopoda these are seen typically amongst the Foraminifera and Radiolaria. The shells may be strengthened by the adhesion of granules of sand, spicules, or other material. In the Foraminifera the shells are external coverings, the pseudopodia being protruded through an opening as a snail emerges from its shell (Fig. 8). In the Radiolaria the skeletal supports are more complicated, and consist of spherical or asymmetrically formed fenestrated shells, strengthened by various radially or tangentially arranged spicules embedded in the cytoplasm (Fig. 78). The cup-like loricæ found amongst the Mastigophora (Fig. 18, 1) and Ciliata (Fig. 23) may be

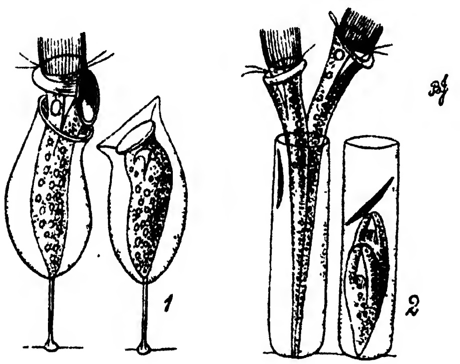


FIG. 23.—CILIATES WITH LORICÆ AND OPERCULA WHICH CLOSE THE ORIFICE WHEN RETRACTION OCCURS (× 250). (FROM LANKESTER, 1903, AFTER KENT AND WRIGHT.)

1. *Cothurina affinis*. 2. *Cothurina valvata*.

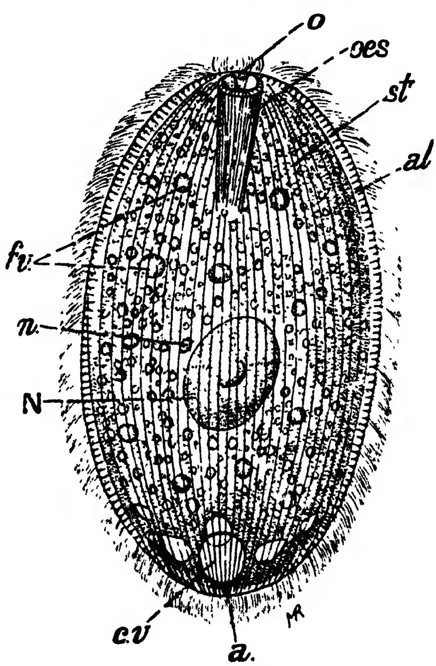


FIG. 24.—*Prorodon teres* (× 660). (FROM MINCHIN, 1912, AFTER SCHEWIAKOFF, 1896.)

N, Macronucleus; *n*, micronucleus; *o*, mouth; *œs.*, œsophagus with rod-like supports; *f.v.*, food vacuoles; *c.v.*, contractile vacuole; *al*, alveolar layer; *st*, meridional rows of cilia; *a.*, anal opening.

regarded as external skeletons or supports. These various structures are secreted by the cytoplasm, from which they are separate. In all Protozoa which have a distinctive body form it is the rigidity of the ectoplasm which enables the organism to retain its shape. In certain cases, what may be regarded as modifications of the cytoplasm are developed for purposes of support. Thus, in certain Ciliata, as, for instance, *Prorodon*, the anteriorly placed cytostome leads to a cytopharynx which is supported by a series of longitudinally arranged rods (Figs. 24 and 25). These rods, or trichites, can be drawn apart and the cytostome opened by radially arranged contractile fibres attached to each rod. In connection with the cytostome of certain Mastigophora, such as *Chilomastix*, the margin of the cytostomal groove which leads to the cytostome is supported and rendered rigid by special fibres (Fig. 69). In *Trichomonas*, again,

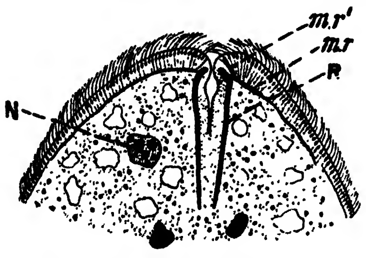


FIG. 25.—SECTION THROUGH CYTOSTOME OF *Prorodon teres*, SHOWING SUPPORTING RODS (× *ca.* 600). (FROM MINCHIN, 1912, AFTER MAIER.)

N, Nucleus; R, rods; *m.r.* and *m.r.'*, myonemes.

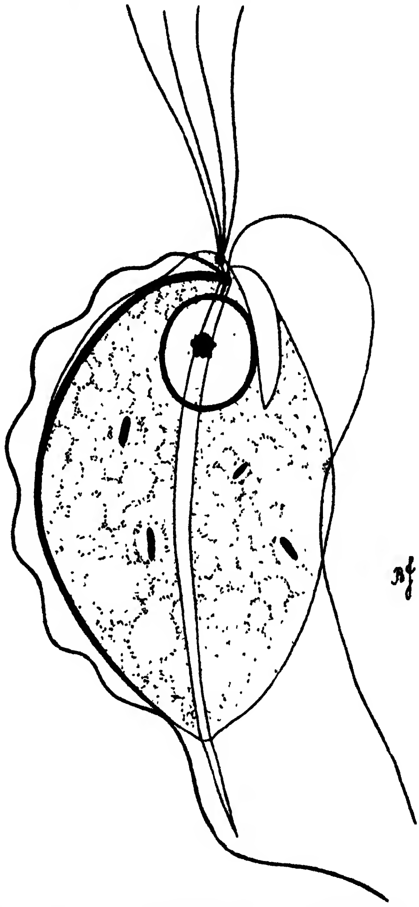


FIG. 26. — *Trichomonas* (*Pentatrichomonas*) FROM THE HUMAN INTESTINE (× 3,200). (AFTER KOFOID AND SWEZY, 1924.)

the line of attachment of the undulating membrane is supported by a special basal fibre which takes origin in a blepharoplast, and appears to function by keeping the membrane stretched to its full extent (Fig. 26).

Another structure which also occurs in *Trichomonas* and allied forms is the axostyle. This is a stiff rod which commences in the blepharoplasts,

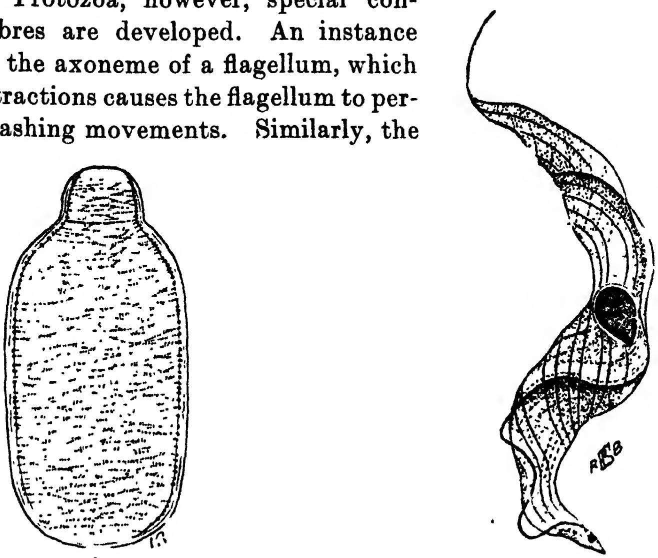
and passes through the centre of the body to protrude with a sharp point at the posterior end. It is a structure which has little affinity for stains, and its function and origin are not properly understood. Sometimes the flagellates are seen attached to débris by the pointed extremity of the axostyle, but this is possibly only accidental. It is, perhaps, best to regard the organ as skeletal in nature. Not infrequently, as explained above, some of the axonemes which arise from the blepharoplasts at the

FIG. 27.—*Trichomonas vaginalis*, SHOWING TENDENCY OF AXOSTYLE TO SPLIT INTO A SERIES OF FIBRILS (× *ca.* 2,000). (AFTER REULING, 1921.)

anterior end of the body, instead of becoming free flagella at the anterior end, pass backwards through the cytoplasm to become free flagella at other parts of the body surface. This condition is well seen in *Hexamita* and *Giardia* (Figs. 288 and 291). It is customary to speak of the intracytoplasmic portions of the axoneme in these flagellates as axostyles, but this is clearly a misapplication of the term, for there is no evidence that the axostyle of *Trichomonas* has any real homology with an axoneme, though Kofoid and Swezy (1915) have suggested that it represents an

intracytoplasmic flagellum. The axostyle usually appears as a clear homogeneous structure, but sometimes a fibre has been described as passing along its central axis, while Reuling (1921) has noted that the axostyle of *Trichomonas vaginalis* may sometimes split into four separate fibrils which originate in the blepharoplasts. He regards the axostyle as composed of four united fibres (Fig. 27).

MYONEMES.—It may be accepted that one of the characteristics of cytoplasm is its power of spontaneous movement. In many Rhizopoda and Mastigophora there are no visible structures which will account for this movement, which involves a relatively large expenditure of energy. In many Protozoa, however, special contractile fibres are developed. An instance in point is the axoneme of a flagellum, which by its contractions causes the flagellum to perform its lashing movements. Similarly, the contractile fibres in the filaments of attachment of certain ciliates, like *Vorticella*, enable the organisms to withdraw themselves suddenly (Fig. 19). In many of the larger trypanosomes, gregarines, and ciliates there are developed in the ectoplasm a series of fibres of a contractile nature known as *myonemes* (Fig. 28). These run in various directions, and by their contractions the organisms are able to perform movements of flexion and extension. They not infrequently give rise to a longitudinal marking of the surface of the body. A common type of movement seen typically in gregarines and merozoites of Sporozoa is the formation of rings of constriction, which pass as peristaltic waves along the body. In certain ciliates para-



A B

FIG. 28.—MYONEMES IN GREGARINE AND TRYPANOSOME. (FROM MINCHIN, 1912, AFTER SCHNEIDER AND MINCHIN.)

A. *Clepsidrina munieri*. B. *Trypanosoma percæ* (× 2,000).

sitic in the rumen of cattle, such as some of the complicated forms like *Diplodinium* (Fig. 520), the anterior region of the body is highly developed, while in association with this there is a complicated system of contractile fibres which enables the organisms to withdraw the whole anterior ciliated region of the body into a depression, which becomes closed over it. In a similar manner the ciliated peristomal region of *Vorticella* and its allies can be suddenly retracted or withdrawn. The curious elongate ciliate *Spirostomum* is well supplied with longitudinal myonemes, which enable it to retract suddenly to the globular form when stimulated (Fig. 509).

The presence of these myonemes often renders it extremely difficult to obtain satisfactorily fixed specimens in the fully expanded condition, as stimulation of the fixing fluid causes immediate contraction of the myonemes, and consequent rounding up of the body.

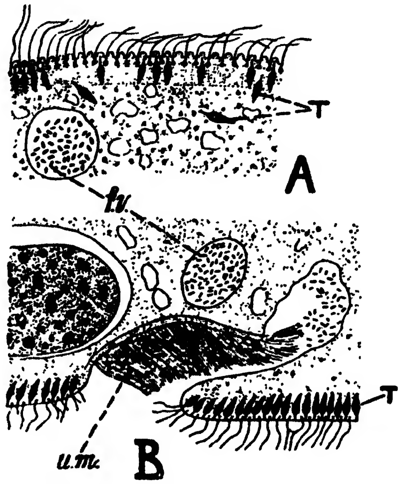


FIG. 29.—TRICHOCYSTS AS SEEN IN SECTIONS OF *Paramecium caudatum* (× *ca.* 1,500). (FROM MINCHIN, 1912, AFTER MAIER.)

A. Body Surface.
B. Mouth and œsophagus.

T. Trichocysts; *f.v.*, food vacuoles; *u.m.*, undulating membrane formed of fused cilia in the œsophagus.

EXTRUSION FILAMENTS.—In some Protozoa special structures occur which, on stimulation, have the property of discharging filaments of varying length. These may be protective or aggressive in function or serve the purpose of fixation.

As organs of protection they are known as *trichocysts*, and are found amongst the Ciliata such as *Paramecium*, *Prorodon*, *Dileptus*, and many other forms. They appear as minute ovoid bodies embedded in the ectoplasm (Fig. 29). From the blunt end there arises a fine process which extends as far as the pellicle or outer layer of the ectoplasm. When stimulated, the fine process is ejected as a tapering filament. Several explanations of the sudden formation of the filament have been suggested. One is that a very rapidly coagulating fluid is discharged. Whether this is the correct explanation or not, it does not appear that the filament as such exists in the trichocyst before it is visible externally. A larger organ with a similar function is the Nessel's capsule or nematocyst. It is present in *Epistylis*, and is arranged in pairs (Fig. 529).

Another type of filament which can be suddenly discharged occurs in the Cnidosporidia (Fig. 30). In this group the resistant cysts or spores are provided with one or more *polar capsules* from which long filaments,

sometimes fifty or a hundred times as long as the spore itself, can be extruded. The spores of the Cnidosporida are developed in a complicated manner from a group of cells, some of which form the polar capsules. These possess a tough outer covering within which the coiled-up filament

FIG. 30.—DARK FIELD VIEW OF SPORE OF *Nosema apis* WITH EXTRUDED POLAR FILAMENT (× 1,200). (AFTER KUDO, 1921.)

can be seen. It is supposed that the filament is inverted in the capsule, and that it is discharged by pressure from within, just as an inverted finger can be everted by blowing into a glove (Fig. 312).

ENCYSTMENT AMONGST THE PROTOZOA.

The majority of Protozoa under certain conditions which are generally adverse to their continued existence, or in anticipation of such conditions, are able to enclose themselves in resistant capsules of varying degrees of impermeability. Encystment is effected by the secretion from the surface of the body of a substance which quickly hardens in the medium. In the majority of instances, while this is taking place, the organism, which has become contracted to a spherical form, revolves slowly, so that fresh secreted material is applied regularly to the layer already formed. These capsules are known as *cysts*, and are generally composed of a clear, hyaline, transparent substance. In free-living Protozoa which live in water encystment takes place when the medium is drying up, and there is danger of desiccation. In this manner complete drying is prevented, and survival for long periods may occur in conditions under which it would be impossible for the exposed organism to live. It has been shown that the sand of the desert exposed to the tropical sun contains encysted Protozoa, which emerge from their cysts when brought into more favourable surroundings. The cysts are usually perfectly smooth on their outer surface, but sometimes they are roughened by the formation of tubercles or other markings. Very frequently, after the resistant cyst has been formed there is secreted a membranous inner lining to the cyst. In such cases one can distinguish a resistant *epicyst* from a more delicate *endocyst*. Sometimes cysts are provided with pores, several of which are present

in those of *Dimastigamœba gruberi* (Fig. 120). To prevent drying of the contents of the cyst, such pores are closed by plugs of some material formed by the cytoplasm. They probably facilitate emergence from the cyst.

Those Protozoa which are able to contract during life to the spherical form produce spherical cysts, but others become encysted without changing their shape to any extent. Thus, the species of *Giardia* produces ovoid cysts, while species of *Chilomastix* and *Embadomonas* cysts which are pear-shaped (Figs. 293, 255 and 256). The cysts (oöcysts) which are formed round the zygotes of various species of *Eimeria* are frequently ovoid in shape, while those which form round the zygotes of the Gregarinina are generally spindle-shaped (Figs. 350 and 483). Though the majority of Protozoa form cysts at some stage of their development, there are some forms in which cysts have never been observed.

The behaviour of the organism within the cyst varies considerably. In many cases the cysts are purely protective in nature, the organism remaining unchanged in the cyst till circumstances again become favourable to a free existence. The encysted organism escapes from the cyst by its gradual dissolution, or through special pores when these are present. In other cases multiplication takes place within the cyst. In the case of *Entamœba coli*, for instance, the nucleus of the encysted amœba divides repeatedly to produce eight nuclei (Fig. 101). Within the oöcysts of coccidia and gregarines there are produced a varying number of daughter individuals known as sporozoites (Fig. 337). Similarly, within the oöcysts of the malarial parasites on the stomach of the mosquito there are developed very large numbers of sporozoites (Fig. 391). Within the cyst of *Giardia* there are produced two daughter flagellates, while in that of *Prowazekella lacertæ* as many as sixty-four daughter flagellates are formed (Fig. 253). Amongst the ciliates, when cysts are formed, they are usually purely protective in nature, but in some cases, at least, reproduction within the cyst takes place. Thus, the various species of *Colpoda* appear to reproduce only in the encysted condition. The ciliate becomes spherical, and by constant rotation forms a spherical cyst. Within it division into two and then into four daughter ciliates occurs. The cyst is then ruptured and the four young ciliates emerge. They then grow into the adult form, when the process is repeated (Fig. 38).

Cyst formation is a very characteristic feature of parasitic Protozoa. Having adapted themselves to life within another organism, their powers of survival under external conditions have been largely lost, and it is by means of their encysted stages that they are able to pass from one host to another. It thus arises that whenever an organism passes from one host to another in such a manner that exposure to external conditions

must occur, it is the encysted forms which render such a transference to a new host possible. In the case of the intestinal amœbæ, though both free and encysted forms escape from the body, it is only the encysted stages which are able to carry infection to a new host. Even if direct transference of unencysted stages occurred, these would, in all probability, be killed by the digestive fluids of the stomach, which the encysted stages can withstand. In the large group of insect flagellates (Trypanosomidæ), again, it is by means of minute encysted stages passed in the fæces that another insect is infected (Fig. 164).

When encystment is about to occur, very frequently changes take place in the encysting organism. The cytoplasm is freed from all food residues, and in consequence becomes much clearer. Not infrequently the cytoplasm becomes charged with food reserve material, such as glycogen. Sometimes, as in the case of *Entamœba coli* and *Entamœba histolytica*, in preparation for encystment special forms of the amœba which are smaller than the ordinary free individuals are produced (Figs. 96 and 100). These forms, which are preparing for encystment, are known as *precystic* forms. Amongst the Sporozoa encystment is associated with a sexual process. In the case of the gregarines two individuals become enclosed in a cyst (gametocyst), within which each gives rise to a number of gametes (Fig. 465). The gametes unite in pairs, and the zygote thus produced itself becomes encysted in the *oöcyst*, within which it divides into a number of sporozoites. In the case of the coccidia, the zygote is encysted in the oöcyst which is formed either before or after syngamy has taken place (Fig. 337). Within the oöcyst the zygote divides into a number of sporoblasts, which in their turn become encysted in *sporocysts*. Inside the sporocysts the sporoblasts divide into sporozoites.

A special type of cyst is produced by the Cnidosporidia. These are provided with one or more polar capsules from which long filaments can be rapidly extruded. They serve the purpose of anchoring the cysts in the intestine, while the wall is opened for the liberation of the enclosed organism (Fig. 311).

For a long time the resistant encysted stages of the Cnidosporidia, coccidia, and gregarines were known as psorosperms, a name introduced by Johannes Müller (1841) for the spores of Myxosporidiida. The spindle-shaped oöcysts of gregarines were frequently referred to as pseudo-navicellæ, a name first used by von Siebold (1839).

The production of secondary cysts within the primary one occurs occasionally in other groups, as in the ciliates of the genus *Colpoda*. A ciliate may become encysted and undergo a diminution in size within the cyst, and then form a second cyst. The process may even be repeated again, giving rise to three concentric cysts. As stated above, the different

species of *Colpoda* multiply within cysts. The four daughter ciliates usually rupture the cyst and escape (Fig. 38). On the other hand, they may each become encysted within the primary cyst. The process of encystment was probably first developed for purely protective purposes, but various reproductive processes have become associated with it. It must be remembered, however, that encystment is not essential to either of these processes, as they frequently occur quite apart from any encystment whatever.

In the majority of cases, when once formed, a cyst undergoes no change in size or shape, though it may gradually increase in thickness. The cysts of parasitic forms are ruptured or dissolved by the action of the digestive fluids of a new host. In some cysts, however, there are formed special pores which are kept closed by a plug of material which is more easily dissolved than the rest of the cyst. Amongst the Sporozoa such a pore in the oöcyst is termed a *micropyle*, and through it the daughter individuals which have been formed within the cyst emerge (Fig. 337).

It sometimes happens that with growth of its contents the cyst increases in size after it is first formed. This process is well illustrated by the oöcyst of the malarial parasite, which increases enormously in size on the stomach wall of the mosquito (Fig. 391). A similar growth also occurs in the case of the oöcysts of the hæmogregarines (*Hepatozoon*), and the cysts of the flagellate (*Prowazekella lacertæ*), which is parasitic in the intestine of lizards (Figs. 253 and 254). It will be evident that if the thickness of the cyst is to be maintained, there must be constant addition to it of fresh material secreted by the enclosed organism.

The cysts produced by any particular species of Protozoon are usually fairly uniform in size, and possess distinctive features. On this account their characters are of great importance for purposes of identification and classification.

THE PROTOZOAN NUCLEUS.

GENERAL FEATURES.—The nucleus, which is an organized structure containing chromatin, is the most important constituent of any Protozoan cell, as, indeed, it is of all cells. It has been shown that the nucleus is essential to the continuation of life, for individuals which have been deprived of their nuclei, though they may survive and move about for some time, quickly degenerate and die, while portions of the cytoplasm, if they contain nuclei, often survive, regenerate, and continue their existence. It seems probable that all the activities of the cell are governed and regulated by the nucleus, which also appears to be mainly responsible for the transmission of hereditary character.

In some Protista, as, for instance, the bacteria and spirochætes, it appears that there is no definite structure which can be called a nucleus, though a granular material usually identified with chromatin is presumed to fulfil the functions of the organized nucleus. Alexeieff (1924*a*), however, maintains that the granules are not chromatin, but mitochondria. It is an exceedingly difficult matter to give a precise definition of the term *nucleus*, though every biologist knows that it is a definite circumscribed structure containing chromatin, and that it behaves in a well-recognized manner. The fact that it divides when cell division takes place is one of its most important features, but there are other structures in the cytoplasm which behave in a similar manner. The one feature which is not shared by other bodies in the cell is that during the sexual process the nucleus, or one of its descendants, is able to unite with another nucleus. In other words, a nucleus is potentially capable of karyogamy.

The majority of Protozoa possess but a single nucleus, except during the process of multiplication, when two or more may be present. Some forms, however, possess two nuclei during the greater part of their life-history, and are therefore binucleate, while others, again, have many nuclei and are multinucleate. Such binucleate and multinucleate forms may be regarded as individuals in which the nucleus has divided preparatory to division of the body, which for some reason or another has been delayed. In the binucleate and multinucleate individuals the nuclei are easily recognized as being of one type. It sometimes happens that when active multiplication by binary fission is taking place, the rate of division of the nucleus exceeds that of division of the cytoplasm, so that temporary multinucleate stages occur. In the case of *Trypanosoma brucei* and other pathogenic trypanosomes in laboratory animals, when active multiplication is proceeding, individuals with four or even a larger number of nuclei may be seen (Fig. 160). In such cases, however, the condition is quickly rectified by repeated divisions of the cytoplasm without further division of the nucleus. In most, if not in all, cases there arrives a period in the life-history of multinucleate forms when division of the body takes place and uninucleate individuals are produced. This is well seen in *Opalina ranarum* of the intestine of the frog, where the organism is multinucleate during the greater part of its life-history, but in the spring uninucleate individuals are produced (Figs. 448 and 449).

Amongst Euciliata there exists a special type of binuclearity. These Protozoa usually possess two nuclei, which differ from one another not only in size and structure, but also in function. The larger one of the two is known as the *macronucleus*, and the other as the *micronucleus* (Fig. 70). In ordinary division of the organism both nuclei divide, and when the body is split into two parts the two daughter individuals each have two

nuclei. At certain stages in the life-history the macronucleus disintegrates and disappears, while the micronucleus divides into two parts, one of which becomes a new macronucleus. This process of regeneration of the macronucleus occurs most usually in association with the process of conjugation, but may also occur during the course of the ordinary asexual multiplication, when it is known as *endomixis*. The fact that the macronucleus is formed from one of the products of division of the micronucleus is the primary reason why the macronucleus is regarded as a nucleus at all. Furthermore, since the macronucleus disappears during conjugation, and takes no part in the process, it is assumed that the micronucleus is essentially the sexual nucleus, and that the macronucleus is vegetative in function, and governs the metabolism and activities of the cell at other times. Though this may be the case, the absolute proof is difficult to obtain. Apart from the fact that it is small in relation to the size of the body, the micronucleus behaves in every way during the whole life of the ciliate as does the nucleus of an organism, such as a flagellate or an amœba, which possesses no macronucleus. There is little direct evidence that the micronucleus of a ciliate is controlling the metabolism and activities of the cell to a less extent than is the single nucleus of such an organism as an amœba.

It is clear that the macronucleus plays an important part in the economy of the cell, and it is equally clear that it is of nuclear origin, but it does not seem clear that because of its existence the functions of the micronucleus are suppressed or supplanted while it is present. The view which maintains that the micronucleus is purely passive during the asexual life of the organism, and only, so to speak, wakes up to activity during conjugation, while the metabolism of the cell at other times is controlled by the macronucleus, has given rise to the conception of two kinds of chromatin, the one sexual or generative in function and the other vegetative. Amongst the Euciliata the two kinds of chromatin are presumed to be separated in different nuclei, while in other cases the same two elements are supposed to coexist in the single nucleus. It is thought that during the sexual process it is the generative chromatin that functions, the vegetative chromatin having been largely got rid of by so-called reduction or maturation processes. At other times it is the vegetative chromatin which is active, while the generative chromatin, though still present in the nucleus, is passive.

In this connection it is necessary to recall the fact that in the Mastigophora the flagella take origin from a structure called the *blepharoplast*. In its simplest form this consists of a minute homogeneous granule, which appears to be little more than a thickening of that end of the axoneme which is in the cytoplasm. In certain stages of development of some

flagellates the flagella are lost, and a non-flagellate stage is developed. When the flagellate stage is resumed, a new axoneme is developed as an outgrowth from the blepharoplast, which may or may not have persisted.

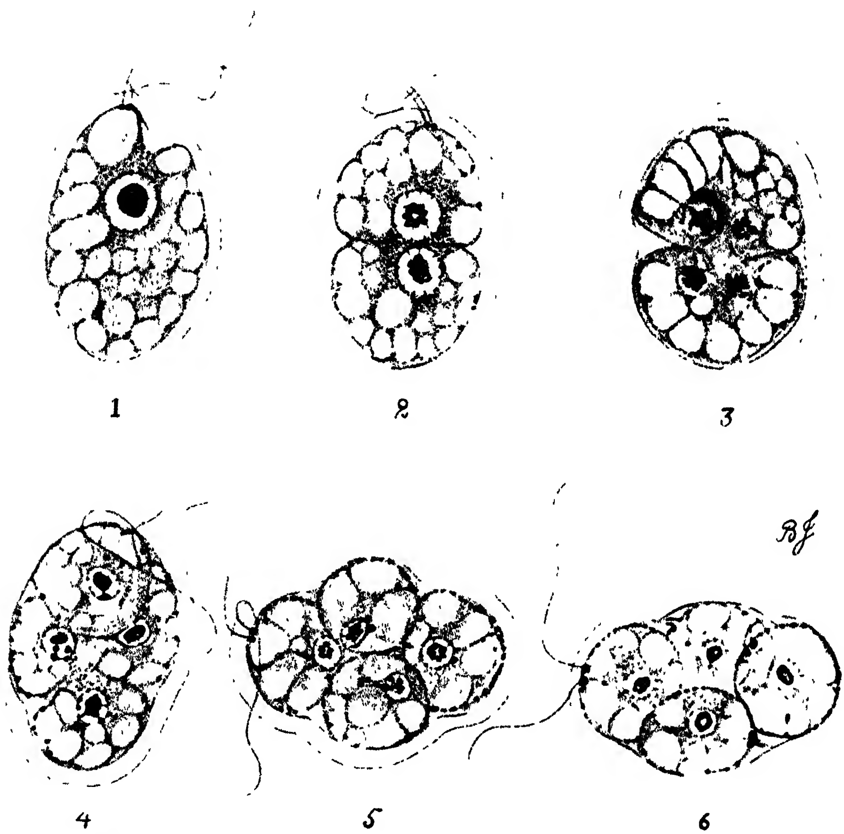


FIG. 31.—*Parypolytoma satura*, TO SHOW THE ORIGIN OF THE BLEPHAROPLAST FROM THE KARYOSOME OF THE NUCLEUS (× 2,600). (AFTER JAMESON, 1914.)

1. Adult flagellate shortly before division.
2. First division completed: two daughter individuals with the old blepharoplasts and flagella.
3. Second division: reconstruction of nuclei and division of body into four.
4. Second division completed: new blepharoplasts are budding off from the karyosome in the upper and left-hand individuals, while the right-hand individual retains the old blepharoplasts.
5. New blepharoplasts on outer surface of nuclear membrane in three of the individuals, while the left-hand individual retains the old blepharoplasts.
6. Stage shortly before release of four daughter flagellates: the left-hand individual has the old blepharoplasts and flagella, while the others have new blepharoplasts and young flagella.

Some evidence has been produced by Jameson (1914) in the case of a flagellate, *Parapolytoma satura*, and by Entz (1918) in the case of *Polytoma uvella*, that when the non-flagellated stages are about to develop flagella new

basal granules or blepharoplasts are developed from the nucleus or from its karyosome (Fig. 31). In the case of *Dimastigamœba gruberi* (Fig. 120), the amœboid phase of which develops flagella under certain conditions, it was stated by Alexeieff (1912*g*) and Wilson (1916) that when this took place the blepharoplasts of the two flagella migrated into the cytoplasm from the karyosome of the nucleus, with which they remained connected by a fibre. As explained below (p. 263), the writer has been quite unable to observe the origin of the blepharoplasts in this manner. It seems more probable that the blepharoplasts are present in the cytoplasm, possibly on the outer surface of the nuclear membrane, during the whole of the amœboid phase

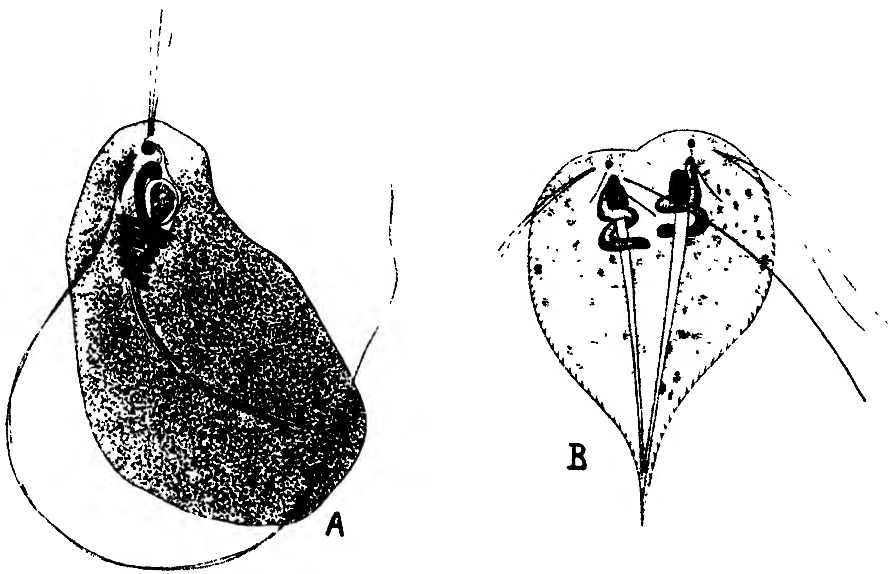


FIG. 32.—*Devescovina striata* (× *ca.* 1,900). (AFTER JANICKI, 1915.)
A. Ordinary flagellate showing coiled parabasal. B. Dividing form.

of the organism, and that they move to the surface of the body when flagella are commencing to form. In many Mastigophora, in association with the blepharoplast, is another structure to which Janicki (1911) has given the name *parabasal* ; it stains intensely with certain stains (Figs. 32, 33, 67). In some cases—as, for instance, in trypanosomes and their allies—it seems to be in actual union with the blepharoplast, and to form with it a composite body, the *kinetoplast* (Fig. 157). There is no conclusive evidence that the parabasal body is of nuclear origin, as some have supposed. It is a well-established fact, however, that division of the organism is preceded not only by division of the nucleus, but also by division of the blepharoplast and parabasal as well, and it becomes a tempting hypothesis

to suppose that the nucleus and the kinetoplast of a flagellate represent two nuclei, as do the micronucleus and the macronucleus of a ciliate. Such an assumption has been made by Hartmann (1907) and others, who regard the kinetoplasts as true nuclei, and the flagellates which possess them as constituting a special group of the Mastigophora, the Binucleata. As pointed out by Alexeieff (1917*b*), there is no ground for this assumption, and in order to avoid confusion he proposed the name kinetoplast in place of kinetonucleus and other terms which implied a nuclear nature. It is safer to regard the kinetoplast as a distinct structure concerned with the activities of the flagellum, even though it divides when cell division occurs, and possibly may have originated from the nucleus in the first place. It might equally be argued that though the macronucleus of a ciliate has originated from the micronucleus, and though it multiplies by division during reproduction, it has ceased to be a nucleus in the true meaning of the term, and has become modified to serve some other purpose, possibly in connection with the development of large numbers of cilia. It is worthy of note that the macronucleus does not divide like a true nucleus, which in most cases, at least, shows some indication of mitosis. Against this view, however, can be raised the argument that there occur certain races of ciliates which possess no micronuclei, though other races of the same species have both micro- and macronuclei. As noted above (p. 25), Dawson (1919) discovered an amicronucleate race of *Oxytricha hymenostoma*, a ciliate which normally possesses both nuclei. The ciliate was kept in culture for several years, and though possessing only a macronucleus, it reproduced regularly by fission.

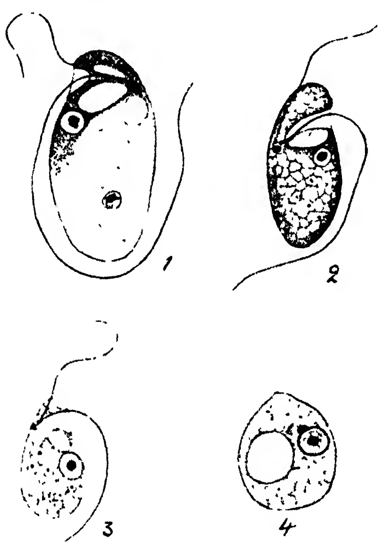


FIG. 33.—*Bodo caudatus* COPROZOIC IN HUMAN FÆCES (× *ca.* 1,500). (ORIGINAL.)

1 and 2. Forms showing two blepharoplasts with associated parabasal.
3. Small individual. 4. Encysted form.

ENDOMIXIS.—As already remarked, the macronucleus of a ciliate degenerates during conjugation, and a new macronucleus is developed from one of the products of division of the micronucleus. This replacement of the macronucleus from the micronucleus may occur at times other than during conjugation. When *Paramecium aurelia* reproduces repeatedly by simple division over long periods at certain

intervals, the macronucleus degenerates, and is replaced from the micronucleus as at conjugation. Woodruff and Erdmann (1914) described the process in *Paramecium aurelia*, and named it *endomixis*. *P. aurelia* contains normally two micronuclei and one macronucleus (Fig. 34). When endomixis occurs, the macronucleus disintegrates and is eventually absorbed. The two micronuclei divide to form four, and these again to form eight micronuclei. Of the four derived from each original micro-

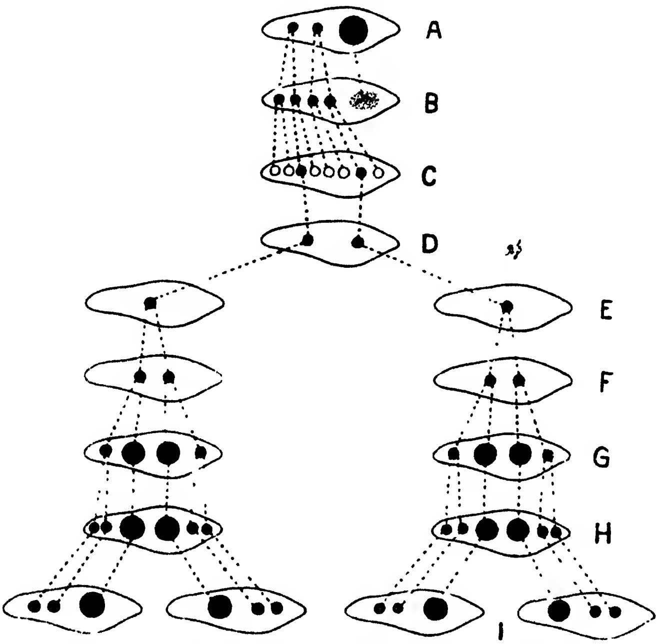


FIG. 34. ENDOMIXIS IN *Paramecium aurelia:* DIAGRAMMATIC REPRESENTATION OF NUCLEAR CHANGES AS DESCRIBED BY WOODRUFF AND ERDMANN, 1914. (AFTER JENNINGS. 1920.)

A-B Degeneration of macronucleus and first division of two micronuclei.
C-D. Second division of micronuclei and degeneration of six of the daughter micronuclei
E Division of ciliate to produce two daughter individuals each with a single micronucleus.
F-G. Two divisions of micronuclei to give rise to four, two of which increase in size to become macronuclei. H. Further division of micronuclei.
I. Division of ciliate to give rise to the normal type as at A.

nucleus, three degenerate, so that two micronuclei are left. The ciliate now divides, giving rise to two ciliates, each with a single micronucleus. In each of these daughter ciliates the micronucleus divides to form two, and again to form four micronuclei. Two of these increase in size and become macronuclei, while the other two divide to form four micronuclei. The ciliate now has two macronuclei and four micronuclei. It now divides to form two ciliates, each of which has two micronuclei and one macronucleus as in the original form.

Endomixis has been demonstrated in several races of *Paramecium aurelia* by Woodruff and Erdmann, as well as in another species of the same genus. Fermor (1913) claimed to have seen the same process in *Stylonychia*, while Calkins (1915 and 1916) described it in *Didinium* and *Uroleptus*. The meaning of endomixis is not clear. That it takes place quite apart from unfavourable conditions has been noted by Woodruff (1925), who also proved that certain races of *Paramecium* in which it did not occur died out. All that can be stated is that for the satisfactory continuation of the functions of the macronucleus, whatever these may be, it seems necessary in many cases that this structure be renewed from time to time. Though this usually takes place during conjugation, it may occur at other times. An exception to this rule is afforded by the behaviour of the ciliate *Spathidium spathula* (p. 132).

STRUCTURE OF THE NUCLEUS.—The nucleus of a Protozoon possesses a nuclear membrane, which may be regarded as a special development of the linin network of fibres or septa which traverse the enclosed space (Fig. 1). The meshes of the network or spaces between the septa are filled with a fluid substance known as nuclear sap. Distributed upon the membrane or network as distinct granules or in one or more larger masses is the chromatin material, while in most cases, somewhere on the network, and most usually at or near the centre of the nucleus, is a body known as the *karyosome*, which, on account of its affinity for certain stains, has generally been regarded as consisting partly of chromatin and partly of an achromatic substance (*plastin*). In some Protozoan nuclei the karyosome does not seem to be present, but it appears in the nuclei of the majority of forms. From what takes place in nuclear division, it appears that the karyosome is composed mostly, if not entirely, of plastin material, and that the chromatin of the nucleus is represented by the granules outside the karyosome, for it is from them that the chromosomes are formed. Doflein (1922), from a study of the nucleus of the flagellate *Ochromonas granularis*, was led to believe that a true karyosome was devoid of chromatin, and that during nuclear division it gave rise to the achromatic part of the spindle, while the chromosomes were derived from the peripheral chromatin which was situated outside the karyosome. On the other hand, Stern (1924), from a study of the nuclear division of the Heliozoon *Acanthocystis aculeata*, arrives at a conclusion which is the exact opposite of this. He believes that the karyosome breaks up and gives rise to the chromosomes, while the spindle is formed from the part of the nucleus between the karyosome and the nuclear membrane.

Sometimes several masses of plastin occur in a single nucleus, but it seems doubtful if these should all be regarded as karyosomes. It is often assumed that an intranuclear centrosome, the centriole, is present in the

nuclei of Protozoa. It is supposed to be embedded in the karyosome, and only to become recognizable as centrosomic in nature during nuclear division, when it divides into two parts which separate from one another, though remaining connected for some time by a fibril, the *centrodesmose.* As the daughter centrioles move apart they take up positions at the ends of the elongating nucleus, while spindle fibres surrounding the centrodesmose may form between them (Fig. 59). The chromatin of the nucleus may form definite chromosomes, which arrange themselves as an equatorial plate at the equator of the spindle. Two daughter plates are formed, and these travel towards the centrioles at opposite poles of the nucleus. When the nuclear membrane divides, the spindle fibres and centrodesmose disappear, a new karyosome is formed around the centriole, and the chromosomes break up into granules, which are distributed on the nuclear membranes or linin network. Observers are, however, by no means convinced that such a granule is a true centrosome, for in many Protozoa undoubted centrosomes exist in the cytoplasm outside the nuclei.

The size of the karyosome in proportion to that of the entire nucleus varies considerably. In many nuclei, especially those of small size, it has been the rule to regard the bulk of the chromatin as being aggregated in the relatively large karyosome, and to suppose that little, if any, is distributed upon the nuclear membrane or linin network. It is becoming increasingly evident, however, that all nuclei contain some chromatin on the linin network or membrane (peripheral chromatin). In other cases the karyosome is relatively small, while definite chromatin granules occur upon the nuclear membrane or the linin network. The nuclei of the first type are often spoken of as of the *karyosome type*, but every transition between the two types of nuclei occurs. This question as to whether a centriole is always present or not is a very difficult one to decide, for the statements regarding it are most conflicting. Some observers are able to find centrioles in nearly every nucleus they examine, while other equally competent observers fail to detect them. The difference of opinion is to be accounted for by the fact that the centriole is merely a minute granule, the nature of which can only be determined by its behaviour during actual division of the nucleus. While division is taking place, there are always numbers of granules in the nucleus. Many of these are chromatin granules, and as spindle fibres are often present while the nucleus is dividing, it is easy to interpret any two granules and a connecting fibre as centrioles and centrodesmose. It thus happens that it is more than doubtful if most of the structures which have been described as centrioles are actually of this nature. The mere presence of a central granule in a karyosome of a resting nucleus appears to some observers to be sufficient

ground for calling it a centriole. Karyosomes do not always stain homogeneously, and may have a granular structure, while the appearance of a central granule may be merely the result of irregular extraction of stain. Before deciding as to the centriole nature of a granule, it is necessary to trace its division and the separation of the two daughter centrioles, and to observe the actual centrodesmose uniting them. Furthermore, when spindle fibres are developed for mitotic division, the daughter centrioles will occupy the poles of the spindle. Such appearances must not be of rare occurrence, but must be detected in the majority of dividing nuclei. When a large number of dividing nuclei of any Protozoon such as an amœba are examined, it is a relatively easy matter to find isolated examples of spindles which have granules at their poles, though the majority of spindles may not show them. The occasional presence of such apical granules does not justify the assumption that they are actually centrioles. It is an undoubted fact that definite spindles may be formed within the nuclei of Protozoa without there being any evidence of centrioles at the poles, though it is not difficult for those who desire to see such structures to convince themselves that granules, which they interpret as centrioles, are present. The fact that during the mitotic nuclear division of the cells of higher animals centrosomes are almost always present, and that undoubted centrosomes occur in some Protozoa, has undoubtedly led to many structures being described as centrioles or centrosomes which have quite another nature. In the present state of our knowledge it is impossible to state that a centrosome or centriole is an essential constituent of all Protozoan nuclei. Nevertheless, it cannot be supposed that all the descriptions which have been given are erroneous. In the division of the nucleus of *Dimastigamœba gruberi*, which has a large central karyosome, the latter structure elongates and becomes dumb-bell-shaped, and finally divided into two parts. As these separate, they remain connected by a fibre which can be shown in many cases to unite two granules which occur in the two daughter karyosomes (Figs. 61, 4, and 120, 12). Whether such granules are to be regarded as intranuclear centrosomes (centrioles) is a question more difficult to decide.

The true structure of a nucleus is that which it possesses in the normal living cell. Fixing fluids and other reagents may considerably alter its appearance, so that the greatest care has to be exercised in the interpretation of the structures seen in fixed material. Experience has shown that certain fixing fluids, stains, and reagents produce better results than others, and are reliable in giving accurate pictures of the true structure. Nevertheless, the literature dealing with the Protozoa, especially the blood parasites which have been largely studied in dried films, is full of erroneous descriptions of nuclei. The dry blood film stained by Romanowsky stains,

though it may give useful information as to the type of cell or parasite present, is completely misleading when it comes to a consideration of the minute nuclear structure. Descriptions of the characters of nuclei which are based on preparations of this kind are not only worthless but misleading.

From the above description it will be realized that the nuclei of Protozoa may be roughly divided into two classes: those in which there is a central karyosome, and those in which no such karyosome is present. Compared with the size of the nucleus, the karyosome may be a relatively small structure, or it may occupy a large part of its bulk. As a type of nucleus with small karyosome, that of *Entamœba histolytica* will serve as an illustration (Fig. 95). There is a definite nuclear membrane, on the inner surface of which practically all the chromatin is arranged in the form of small granules. At the centre of the nucleus is a small granule, the karyosome, which presumably consists of plastin material and possibly some chromatin. Surrounding the karyosome is a clear area, the limits of which form a sphere (or ring in optical section) of fine granules. These do not contain chromatin, but represent the inner limit of the linin network which connects the sphere with the nuclear membrane. The linin network appears to be free from chromatin. Dobell (1919), who has studied the nuclear division in this amœba, could obtain no evidence of the existence of a centriole in the karyosome, though such a structure has been described by Hartmann (1908-1913). During division of the nuclei within the cysts the writer has seen forms which suggest the presence of a central granule which divides (Fig. 57).

A type of nucleus in which a definite and relatively large karyosome is present is of frequent occurrence. It is seen typically in trypanosomes, many free-living amœbæ, and other Protozoa (Figs. 48, 89, 224). These karyosomes are comparatively large structures which are connected with the nuclear membrane by the linin network. It is possible, though by no means certain, that some of the chromatin of the nucleus may be concentrated in the karyosome, which stains intensely with certain nuclear stains. The nuclear membrane and the linin network may have comparatively little chromatin, which in small nuclei, such as those of trypanosomes, is difficult to detect. In many cases nuclei of this type are described as possessing centrioles within the karyosomes. The large karyosome may appear perfectly uniform and homogeneous, or it may show indications in stained specimens of being composed of a varying number of deeply staining bodies embedded in a more faintly staining plastin matrix. The karyosome is often spherical in form, but it may be irregular in shape. In some cases on the surface of the karyosome there occur deeply staining granules, which may be chromatin, while the central part consists of plastin. Sometimes one or more vacuoles are present.

Between these two types of nuclei many intermediate forms are found, and individual variations are of common occurrence. These variations may affect the nuclear membrane, which may be exceedingly fine in some forms and comparatively thick and dense in others. The arrangement of the chromatin upon the membrane may be in the form of uniformly distributed fine granules, or there may be coarse granules more irregularly distributed, or most of the chromatin may be aggregated into a semilunar mass planted on one side of the membrane.

The linin network itself may be in the form of a uniform mesh, or it may consist of radially arranged strands. The meshes of the network may contain granules other than chromatin or globules of an undetermined nature. The minute structure of the nuclei is of considerable importance in the differentiation of species.

Though it is not possible to draw a hard-and-fast line between those nuclei which possess karyosomes and those which do not, there nevertheless exist certain nuclei in which there appears to be no tendency towards the formation of a central structure. Amongst the gregarines, for instance, certain individuals of a particular species may show a single deeply staining body in the nucleus, or more than one, while in some Protozoa there are a series of deeply staining bodies upon the nuclear membrane, while the interior of the nucleus is occupied by a uniform meshwork of fibrils. It appears impossible to speak of several bodies in the nucleus as karyosomes, a term which is undoubtedly used by the vast majority of zoologists, for the single more or less centrally placed structure described above. Nuclei of the type which has no definite karyosome may, however, contain a body which may or may not be central in position, and which is regarded as devoid of chromatin, owing to the fact that it does not stain intensely with chromatin stains. Such a structure occurs, according to Metcalf (1909), in the nuclei of species of *Opalina*, in which a deeply staining karyosome is not present. It resembles the nucleolus which is commonly found in the nuclei of the cells of higher animals. Bodies of this type have also been described by Reichenow (1921) in the nuclei of various stages of development of the hæmogregarines of the genus *Karyolysus*. They are also present in the nuclei of *Hepatozoon balfouri*, and, as in the case of *Karyolysus*, they divide during nuclear division (Fig. 35). They are commonly present in the nuclei of coccidia. They take no part in the formation of the spindle or the chromosomes.

Another type of nucleus which has to be mentioned is the macronucleus of the Euciliata. It has already been explained that these Protozoa typically possess two nuclei—the micronucleus and the macronucleus. The former is usually of the type which contains a central

karyosome, and when it divides it does so by mitosis, while the latter, though developed from a nucleus like the micronucleus, has so changed in appearance and structure that it seems doubtful if it should still be regarded as a true nucleus (Fig. 37). It is sometimes spherical in form, but it is more usually slightly elongated. It may be many times as long as it is broad, and in such cases may have a beaded appearance, as in *Stentor* and *Spirostomum* (Figs. 22 and 509). It may even be irregularly branched, as in certain Suctoria (Fig. 531). It consists of a dense material impregnated with granules which become more evident during division. Vacuoles are often present, while sometimes, as in the species of *Colpoda*, the elongated macronucleus contains within it one or more deeply staining bodies (Fig. 498). It is evident that the macronucleus differs in many ways from the micronucleus and the nuclei of other Protozoa. During division it does not behave as true nuclei do, and there seems to be little change in its appearance, except for the greater clearness of its

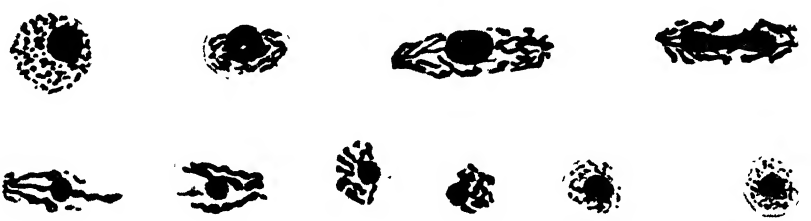


FIG. 35.—STAGES IN THE FIRST NUCLEAR DIVISION IN THE SCHIZONT OF *Hepatozoon balfouri*, SHOWING DIVISION OF THE KARYOSOME, WHICH APPEARS TO BE ENTIRELY DEVOID OF CHROMATIN (×6,000). (ORIGINAL.)

granules. It is generally assumed that the granules in the macronucleus are chromatin, but, if this be so, there must have taken place a remarkable increase in the chromatin during its formation and growth from the micronucleus from which it was originally derived. It seems not impossible that this material is not actually chromatin, but some other substance which has been elaborated to fulfil a special function.

In this connection it will be necessary to refer again to a theory which was suggested by Schaudinn, and subsequently elaborated by Goldschmidt and others. According to this theory the Protozoan nucleus is constructed of two fundamentally different parts, which in the Euciliata are separated in two distinct nuclei. The one part consists of vegetative material which controls nutrition, movement, and other vegetative functions, while the other is composed of generative material which takes part in the syngamic process. This theory has been extended to the chromatin itself, which is supposed to be of two kinds, the one idiochromatin, which takes part in syngamy, and is responsible for the transmission of hereditary

characters, and the other vegetative chromatin, which has to do with the vegetative functions. Amongst the Plasmodroma and the Protociliata both kinds of chromatin are contained in one nucleus, and it is supposed that the extrusion of chromatin material from the nuclei of gametes, which has been described as taking place in certain instances, is an expulsion of the vegetative chromatin in preparation for syngamy. If this explanation is the correct one, it has to be admitted that after syngamy the vegetative chromatin can be re-formed from the generative chromatin, as illustrated by the formation of new macronuclei from the micronuclei after syngamy in the Euciliata. The theory depends very largely on an exact definition of what is and what is not chromatin, and a correct interpretation of the various parts of the nucleus, about which at the present time there is considerable difference of opinion. Dobell (1925) has described a condition of binuclearity in Aggregata (see p. 873).

MULTIPLICATION AMONGST THE PROTOZOA.

Multiplication takes place by a process of binary fission or gemmation in which an organism divides into two daughter organisms after division of the nucleus, or by a process of multiple segmentation, which is generally known as *schizogony* amongst the Sporozoa, where it occurs most typically, after a number of nuclei have been formed by repeated divisions.

BINARY FISSION.—The process of binary fission may give rise to daughter forms which are equal in size (equal binary fissions), or to forms which are unequal in size (unequal binary fission). When there is a marked difference in size between the two, the process is known as budding or gemmation, a method of multiplication which is seen typically amongst the attached Euciliata (Peritrichida and Suctoria), where a large form buds off a small ciliated embryo which does not itself reproduce till it has grown to the adult form.

In the case of amœbæ which have globular bodies, binary fission is effected by the body becoming elongated and a constriction forming around the middle of the body (Fig. 36). This deepens till the amœba is divided into two parts. The daughter forms may not divide again till they have grown to the size of the parent. On the other hand, they may divide before growth is complete, with the result that increasingly small individuals are produced. If they divide only after they have grown to a size larger than that of the parent, then larger forms are gradually produced. In the case of the amœbæ it is evidently impossible to state that division takes place in any one plane, except that it occurs in a plane at right angles to the axis occupied by the elongate dividing nucleus.

Directly it becomes possible to orientate an organism, and state that it possesses an anterior and posterior end and a dorsal and a ventral

surface, it is found that the plane of division is uniform in the different groups. Thus amongst the Mastigophora, which have an anterior flagellated end of the body, it is found that in binary fission the body splits longitudinally from before backwards. In those forms in which a cytostome is present, as in *Chilomastix*, in which a dorsal and ventral surface can be distinguished, it is found that a new cytostome is formed near the original one, and if this is also regarded as being on the ventral surface then the body splits longitudinally from before backwards and in a dorso-ventral plane which passes between the cytostomes. In actual division, however, the body often becomes so distorted that it may be difficult to

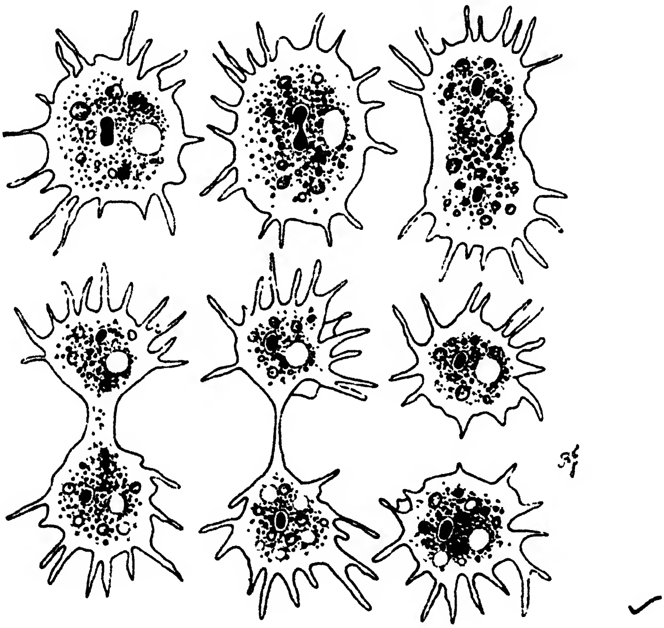


Fig. 36.—Successive Stages in Binary Fission of *Amœba polypodia* (× 250). (From Lang, 1901, after Schulze, 1875, modified.)

distinguish the dorsal and ventral surface, though the plane of division may still be regarded as in this direction. In flagellates such as *Trichomonas* and the trypanosomes, which possess undulating membranes, division is more complicated (Figs. 160 and 271). A new axoneme grows out from the free half of the divided blepharoplast and passes along the border of the membrane. The membrane then divides between the two axonemes, but the point up to which the membrane has divided at any stage is always a short distance behind the end of the new axoneme. When the new axoneme has reached the end of the membrane the division of the membrane is completed, and the two undulating membranes, each

with its axoneme, are formed. The body of the flagellate then divides longitudinally from before backwards in a dorso-ventral plane between the two dorsal membranes. Owing to the blepharoplasts being situated at the anterior end of the body in *Trichomonas* and some other flagellates, the membrane divides from before backwards in these forms. In the trypanosomes, however, the blepharoplast is situated at the posterior end of the flagellate, and the membrane divides from behind forwards. In either case, the body itself divides from before backwards after the membrane has completed its division.

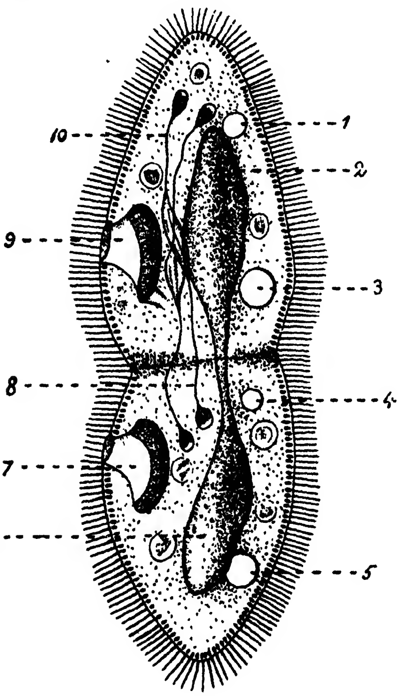


FIG. 37.—BINARY FISSION OF *Paramecium aurelia* (× *ca.* 500). (AFTER LANG, 1901.)

1 and 4, New contractile vacuole; 2 and 6, dividing macronucleus; 3 and 5, anterior and posterior contractile vacuoles, which will become the posterior vacuoles of the daughter forms; 7, new cytostome formed as bud from original cytostome; 8 and 10, mitotic division of two micronuclei.

Multiplication by binary fission occurs also amongst the Opalinata and Ciliata, but division is transverse and not longitudinal, as in the Mastigophora (Fig. 37). A ciliate may develop a new cytostome at some distance behind the first one, and after division of both the macronucleus and micronucleus the body divides transversely or at right angles to the longitudinal axis. It often happens that, as a result of this division, the character of the daughter forms differs from the parent in the relative size of the cytostome. As the body of the new individual is developed from the post-cytostomal region of the parent, it follows that the daughter forms will have a cytostome which is relatively longer when compared with the total length of the body. In *Paramecium* the cytostome of the parent takes up a position at the centre of the body, and is divided into two cytostomes of equal or unequal length, after which the body divides transversely between the two.

Binary fission, when it occurs amongst the Rhizopoda, Mastigophora, or Ciliophora, usually gives rise to individuals which are roughly equal in size; but not infrequently, as, for instance, in *Trypanosoma lewisi*, a large trypanosome will divide in such a manner as to give rise to one large form and one which is very much smaller (Fig. 197, 5). The process is repeated

by the large form, which apparently has ceased to grow, so that eventually its entire cytoplasm is used up in the production of a number of small forms. At each division there is a nearer approach to equal binary fission. It is evident that such a method of division approaches a budding process.

Binary fission usually occurs in the free-living state, and as the division is taking place the organism may be actively motile. Amongst the Rhizopoda, the amœbæ are frequently perfectly quiescent while binary fission is proceeding. In some cases, binary fission takes place in the encysted condition. This appears to be the normal method of multiplication of species of *Colpoda*. The organism secretes a cyst in which it

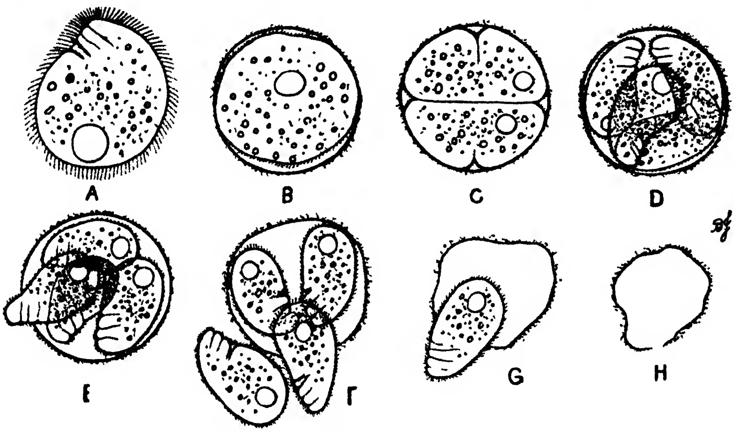


FIG. 38.—*Colpoda steini*: MULTIPLICATION OF A SINGLE INDIVIDUAL DURING A PERIOD OF SEVEN HOURS' OBSERVATION (×650). (ORIGINAL.)

A. Ciliate about to encyst. B. Encysted ciliate.
C. Division into two completed: commencing division into four.
D. Four daughter ciliates in cyst.
E-G. Escape of ciliates through rupture in cyst wall.
H. Crumpled cyst after escape of ciliates.

divides into two, each of which again divides (Fig. 38). The four daughter ciliates then rupture the cyst and swim away. Similar divisions within cysts occur amongst the Rhizopoda and Mastigophora (Fig. 143).

SCHIZOGONY.—By this term is understood a method of multiplication which occurs typically amongst the Sporozoa (Fig. 39). As the organism is growing, repeated divisions of the nucleus and daughter nuclei take place, till finally there may be present a large number of nuclei in a single mass of cytoplasm. The number of nuclei produced varies considerably, and may be as few as four or as many as a hundred or more. The nuclei arrange themselves on the surface of the cytoplasm, which becomes raised into a series of elevations, into each of which a nucleus

passes. When the requisite quantity of cytoplasm has been raised into the elevation this is divided off by a constriction, and the daughter forms, termed merozoites, are produced. These grow into adults, which may again reproduce by schizogony. It is often supposed that the multinucleate adult, which is termed a *schizont*, suddenly segments into a

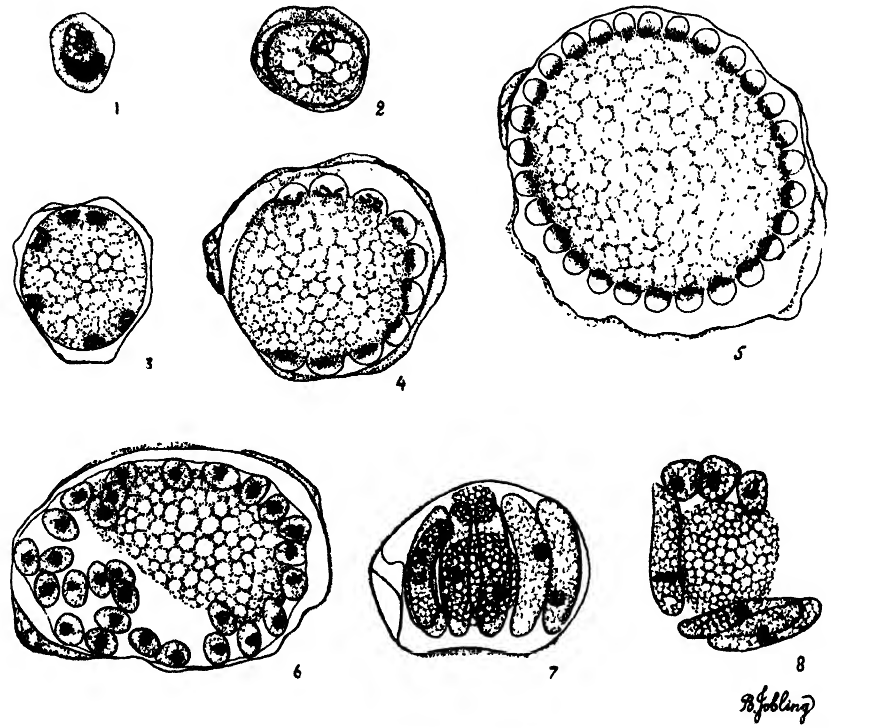


FIG. 39.—*Hepatozoon canis:* DEVELOPMENTAL STAGES IN THE SPLEEN OF A BAGDAD DOG (×2,000). (ORIGINAL.)

1. Young schizont in mononuclear cell. 2. Slightly older schizont.
3. Section of an older schizont with a number of nuclei.
4. Section of a schizont in which merozoites are commencing to form by a budding process.
5. More advanced stage of budding process as seen in section of mature schizont.
6. Merozoites and residual body after schizogony: the merozoites are gametocytes which enter the mononuclear cells of the blood-stream.
7. Section of stage with eight large merozoites which are probably destined to become schizonts again. 8. Stage similar to that depicted at 7.

number of merozoites, as in the case of malarial parasites. It appears, however, that in all cases the merozoites, whether few in number or more numerous, are formed as small buds at the surface of the schizont, as described above. A variable quantity of the cytoplasm is unused in the formation of the merozoites. This residual cytoplasm, within which may be found a certain number of unused degenerate nuclei of varying size,

and any other material to be discarded, such as pigment, is known as the residual body. It takes no further part in the life of the organism, and after separation of the merozoites quickly disintegrates (Fig. 39, 6).

Amongst the Sporozoa, after syngamy has taken place, the zygote divides by a process which is essentially the same as schizogony. This is termed *sporogony*, and it gives rise to *sporozoites*, which differ in size and shape from the merozoites. The sporozoites arise from the multinucleate zygote, which may have increased considerably in size and is called the *sporont*, by a budding process which is very similar to that by which the merozoites are formed (Fig. 455). The term "sporogony" is generally extended to include the whole phase of the developmental cycle from the beginning of the production of gametes or gametocytes to the formation of sporozoites from the sporont after syngamy has taken place. To distinguish the other phase of development during which schizogony occurs repeatedly without the intervention of a sexual process, it has been termed *agamogony*, and the various stages (merozoites and schizonts) *agamonts*. The growing agamont is often termed a *trophozoite*.

During the formation of merozoites and sporozoites it not infrequently happens that the number of nuclei present is so large that the surface of the cytoplasm is insufficient to accommodate them all. By a process of vacuolation of the cytoplasm the available surface is increased. The vacuoles may open into one another, so that the cytoplasm is reduced to the condition of a coarse network. In this way the available surface upon which nuclei can take up their position is increased, so that the merozoites or sporozoites can be budded off in the usual manner. A typical instance of this increase in surface occurs during the formation of sporozoites in the oöcysts of the malarial parasites on the stomach of mosquitoes, as also during schizogony of *Aggregata eberthi* and other Sporozoa (Figs. 377 and 391).

A method of schizogony which occurs amongst the piroplasmata must be mentioned. In these parasites the number of daughter forms produced are two or four, which are described as arising from the parent by a budding process, in contrast to the supposed segmentation of the schizont of the malarial parasites. As already explained, the merozoites of malarial parasites are not produced from the parent by a sudden splitting of the body between the nuclei, but by the formation of buds from its surface, as occurs generally amongst the Sporozoa. The piroplasmata are no exception to this rule. In some species (*Babesia canis*) the buds are usually two in number, but may be four (Fig. 417). In others (*B. equi*) there are usually four buds, as in *Plasmodium minasense* (Fig. 416 and Plate XVII., 6-15, p. 982). The bud commences as a small cytoplasmic elevation on the surface of a rounded parasite. It gradually increases in

size at the expense of the cytoplasm of the parent. It is difficult to understand why an organism which is to produce only two daughter forms should do so by a budding process instead of by a simple binary fission into two parts. It seems possible that it is a condition which has evolved from one in which a larger number of merozoites were originally produced, as in typical schizogony.

When a schizont is in process of producing merozoites or a sporont sporozoites, the schizont or sporont may first divide into a number of intermediate bodies which actually produce the merozoites or sporozoites. In the case of the coccidium *Caryotropha mesnili*, when about sixteen nuclei are present in the schizont, it divides into sixteen portions which have been called *cytomeres* or *agametoblasts* (Fig. 375). The nuclei of these undergo further divisions, and finally merozoites are budded from their surfaces. A similar method of multiplication occurs in *Klossiella cobayæ* and other forms (Fig. 449). Similarly, during sporogony the zygote, instead of dividing directly into sporozoites, may first produce a number of *sporoblasts*, which give rise to the sporozoites. In the coccidia sporogony takes place within the oöcyst which has formed around the zygote, and it frequently happens that the sporoblasts secrete around themselves secondary cysts or *sporocysts*, within which the sporozoites are finally produced (Fig. 337).

Attention has already been called to the fact that occasionally, amongst flagellates which normally multiply by binary fission, the rate of division of the nuclei may exceed that of the cytoplasm during very rapid multiplication, so that stages are reached in which an abnormal number of nuclei are present (Fig. 142). The excessive nuclear multiplication, however, comes to an end, and the body divides repeatedly till a number of normal uninucleate forms are produced. In some cases such multinucleate stages occur normally in the developmental process. Thus, in the course of the development of *Trypanosoma lewisi* in the flea, the trypanosomes taken up from the rat enter the cells lining the stomach, and there grow into large bodies which possess as many as sixteen nuclei, kinetoplasts, and flagella (Fig. 200). The "sphere," as it is called, then divides into a corresponding number of trypanosomes. Such a method of multiplication is really one of delayed division of the cytoplasm, and must be distinguished from true schizogony. It seems probable that the final division of the "sphere" takes place by repeated binary fissions.

During the process of schizogony the merozoites produced by any particular organism vary as regards size and numbers. In certain cases the variations are at a minimum, as, for instance, amongst the human malarial parasites. *Plasmodium malariæ* of quartan malarial fever pro-

duces nearly always eight merozoites, and these vary little in size (Plate XIII., p. 934). Similarly, *Plasmodium vivax* of benign tertian fever produces, as a rule, sixteen, but departures from this number are not uncommon (Plate XII,, 16-18, p. 926). Amongst other Sporozoa, however, greater variations occur, as will be described below. In some cases it has been supposed that the schizogony was of two types—the one giving rise to a small number of large merozoites, and the other to a large number of smaller ones (Fig. 39, 6 and 7). It was supposed that this represented a sexual dimorphism, one line ending in gametocytes of the female sex, and the other in gametocytes of the male sex. More careful study of such cases has thrown doubt on these conclusions, and has tended to show that every transition, both as regards numbers and size, occurs between the two types. Thus, in the case of *Adelina dimidiata*, a coccidium of the centipede, the merozoites produced by a schizont vary in number from four to sixteen, as demonstrated by Schellack (1913). As a rule, when the number is large the merozoites are small, and *vice versa*. In *Hepatozoon canis* (Fig. 39) the number of merozoites produced may be only four, or it may exceed a hundred. In this case it appears that with successive schizogony the number of merozoites produced increases, while their size diminishes, till finally there are formed a large number of small ones which enter the leucocytes and become gametocytes. It has thus to be remembered that in any individual species the merozoites produced at schizogony may vary considerably, both in number and size.

In the case of sporozoites which are produced from the zygote by a process similar to schizogony, the number and size is much more constant. Thus the zygotes of coccidia belonging to the genus *Eimeria* invariably produce eight sporozoites which are contained in pairs in four sporocysts (Fig. 337). In other cases, as, for instance, in the genera *Barrouxia* and *Aggregata*, though the number of sporocysts produced by the zygotes of any particular species may vary considerably, the number of sporozoites in the sporocysts is constant (Fig. 376). On account of its uniformity the type of sporogony is of greater value for purposes of identification and classification than are the forms observed at schizogony.

GEMMATION OR BUDDING.—By this method of reproduction is to be understood one in which an organism, after its nucleus has divided, instead of splitting into two equal or nearly equal parts, divides very unequally, so that one daughter form is very much smaller than the other. The condition is one of extreme unequal binary fission. It is usual to regard the large form as a parent individual, and the small one as a daughter. The process has been described as occurring in free-living amœbæ, and the unequal divisions seen in *Trypanosoma lewisi*, which has already been referred to, may be regarded as an instance of gemmation (Fig. 197). It

occurs, however, most typically amongst the Euciliata in the attached Peritrichida like *Vorticella*, and amongst the Suctoria. In many species of *Vorticella* and allied forms the body divides into two equal parts, so that two equal-sized individuals are attached to the end of a single stalk. One of these may escape and, attaching itself, develop a new stalk, or it may remain attached, and the two individuals may form new stalks, so that eventually a complicated system of dichotomous branches is produced. The division, though apparently longitudinal, is really transverse, as will be evident if it is remembered that the organisms are attached to the stalks by their dorsal surfaces. In some cases the division of the body is unequal, so that a very small individual is separated from a large one. These small forms are provided with circlets of cilia, by means of which

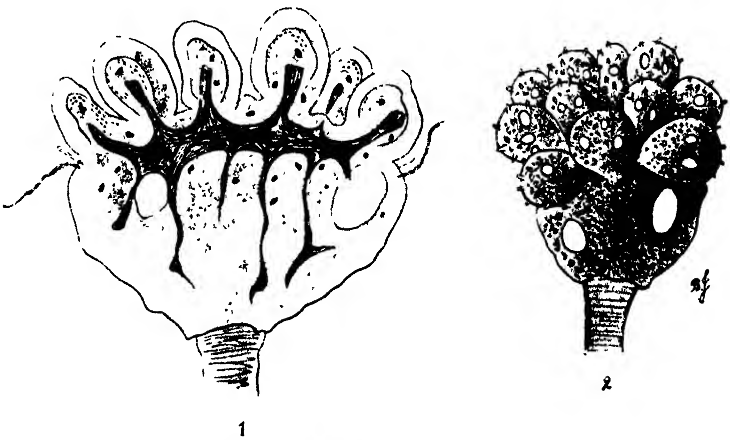


FIG. 40.—*Ephelota gemmipara* IN GEMMATION (× *ca.* 350). (AFTER COLLIN, 1912.)

1. Section of an entire organism, showing method of budding of the macronucleus to form nuclei of buds. 2. Surface view of budding individual.

they swim away, and ultimately conjugate with one of the larger attached forms (Fig. 44).

In the Suctoria buds are formed, either from the surface of the body or in cup-like depressions. In *Ephelota*, studied by Hertwig (1876), the nucleus becomes much branched, and as buds are formed on the surface of the body, portions of the macronucleus enter each bud. The buds are finally separated as ciliated embryos (Fig. 40). In other cases, as in *Tokophrya* and *Choanophrya*, there occurs a process of internal budding (Fig. 532). A depression is formed in the cytoplasm, and the margins of this close to include a space which communicates with the exterior by a pore. A bud is formed from the surface of the cytoplasm within this space. A ciliated embryo is detached, and eventually escapes through the pore.

Though the daughter individuals formed at binary fission may be so

unequal in size that the process is regarded as one of budding or gemmation, the nucleus of the bud arises by equal division of the nucleus of the parent, so that the large and small daughter forms have their nuclei of equal size. A method of formation of the nuclei of buds from chromidia has been described as occurring in certain Protozoa. Thus, in the case of *Entamœba histolytica*, Schaudinn (1903) supposed that granules of chromatin occurred in the cytoplasm outside the nucleus. These granules were supposed to collect in groups at the surface of the organism, become organized into nuclei, and enter the buds which were forming. Such a process certainly does not occur in *E. histolytica.* Another instance in which nuclei have been described as arising in this manner is that of *Arcella vulgaris* referred to above (Fig. 2).

SYNGAMY AMONGST THE PROTOZOA.

As in the higher animals and plants, at certain phases of development, two cells unite and their nuclei fuse, so amongst the Protozoa a similar process may occur. This is generally known as a sexual process, or *syngamy.* It may take place in one of two ways: either two individuals, which are known as *gametes*, unite by fusion of their cytoplasm, followed by union, or *karyogamy*, of their nuclei; or two individuals become incompletely united, and part of the nucleus of each passes over into the other individual to unite with its nucleus. After this transference of nuclear material the individuals separate. The process in which two individuals unite completely is known as *copulation*, while that in which interchange of nuclear material between two temporarily associated individuals takes place is called *conjugation.* The two processes are not essentially different from one another, for it may be considered that in conjugation each of the two associated individuals really produces two gametes, one of which is large and contains all the cytoplasm and a nucleus, while the other is small and consists of a nucleus only. The small gamete produced by one individual unites with the large gamete produced by the other. It is, however, convenient to distinguish the process of copulation from that of conjugation, as the latter is the characteristic method of syngamy amongst the Euciliata.

COPULATION.—This process consists in the union of two cells with fusion of their nuclei. The cells are known as gametes, while the single uninucleated cell resulting from the union is called a zygote, and the nucleus of the zygote, which is the product of the union of two gamete nuclei, is the synkarion. The uniting gametes may be the ordinary individuals which have ceased multiplying, or an ordinary individual, by a special process of multiplication, may give rise to a number of smaller gametes

which unite in pairs. In the latter case, the individual which gives rise to the gametes is known as a *gametocyte*, and the process by which it gives rise to the gametes as *gametogony*.

The gametes which unite may be alike in size and shape, in which case they are known as *isogametes*, and the process of union as *isogamy*. On the other hand, they may be recognizably different from one another in size or structure, and are known as *anisogametes*. The process is then called *anisogamy* or *heterogamy*. If the gametes differ in size, the large

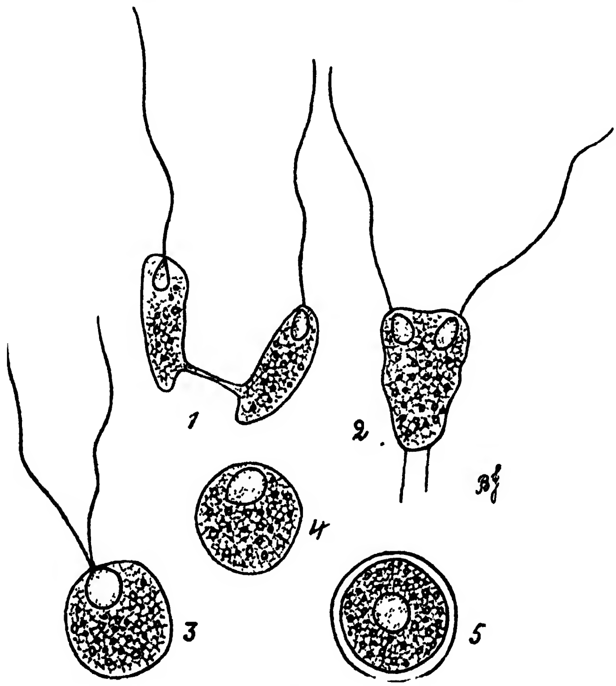


FIG. 41.—SYNGAMY OF *Cercomonas longicauda* (× *ca.* 2,000). (AFTER WOODCOCK, 1916.)

1. Two individuals uniting by their posterior ends.
2. Later stage in the union.
3. Still later stage after nuclei have fused.
4. Stage in which flagella are lost and body rounded.
5. Encysted zygote.

one is called the *macrogamete* and the small one the *microgamete*. It usually happens that the small gamete or microgamete is actively motile, on which account it is regarded as the male gamete, as it corresponds in function with a spermatozoon of higher animals. The larger macrogamete, which is usually a passive body heavily charged with food reserve material, corresponds with the ovum. There is every transition between the process of isogamy and anisogamy. Thus, in some cases the gametes are equal in size, but differ from one another only in the size of their nuclei. In other

cases one gamete is only slightly larger than the other, and there is every gradation towards forms like coccidia or malarial parasites, in which the macrogamete is a comparatively large cell and the microgamete a very minute one.

As an illustration of syngamy in which two ordinary individuals unite, the case of *Cercomonas longicauda*, as described by Woodcock (1916), may be considered (Fig. 41). Two flagellates of the ordinary type come together and unite by their posterior ends, the union gradually extending forwards. After the two flagellates are completely fused their nuclei unite to form a synkarion. The zygote which is produced may commence multiplying by binary fission in the usual manner, or it may encyst. A similar process occurs in *Polytoma uvella*, but is modified as a result of the protective covering of the body (Fig. 42). Two flagellates unite by their anterior ends, and the cytoplasmic contents of one flow into the other,

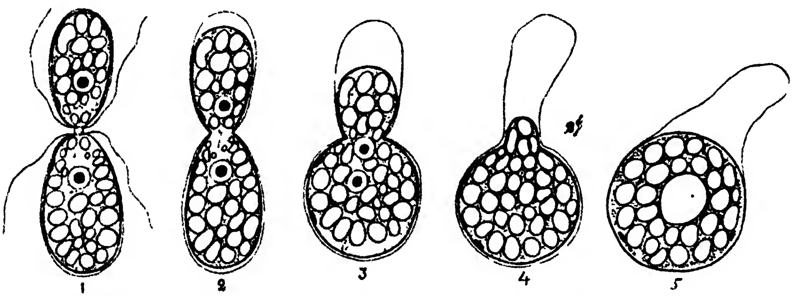


FIG. 42.—*Polytoma uvella:* THE PROCESS OF ISOGAMY AS OBSERVED DURING THE COURSE OF THREE HOURS (× 1,500). (ORIGINAL.)

The contents of one flagellate flow into the other, which gradually becomes spherical. Finally, a cyst is formed after complete fusion has occurred. The nuclei were no longer visible in the later stages. The dark rods are the stigmata.

giving rise to a spherical zygote which becomes encysted. In the case of *Copromonas subtilis* two individuals fuse completely, and Dobell (1908*b*) has stated that each nucleus before union gives off two reduction bodies (Fig. 48). All these instances are cases of isogamy, in which the gametes differ little, if at all, from the ordinary adult individuals. Isogamy has been described by Woodcock (1916) for *Spiromonas angusta* and *Copromonas ruminantium*.

In most cases, however, certain individuals termed gametocytes, which may differ from the ordinary reproducing forms, by a special type of multiplication (*gametogony*) give rise to a number of gametes, which then unite. Syngamy of this type occurs amongst non-parasitic Protozoa, and has been described, in the case of Foraminifera (*Trichosphærium*), Radiolaria (*Thalassicola*), and other forms, but the best-known instances occur amongst parasitic Sporozoa. In the reproduction of *Monocystis magna*,

a gregarine of the earth-worm, Cuénot (1901) described the process of syngamy. Two individuals (gametocytes) encyst together in a common cyst (gametocyst), and each gives rise to a large number of gametes which appear to be completely alike (isogamy). The gametes produced by one individual unite with those produced by the other. The zygotes thus formed become encysted in secondary cysts (oöcysts). In his description of syngamy in *Monocystis rostrata*, another gregarine of the earth-worm, Muslow (1911) also found that there was complete isogamy.

From this condition of complete isogamy, various transition stages leading to marked anisogamy are known amongst gregarines. Thus, in the case of *Lankesteria ascidiæ*, Siedlecki (1899), and in the allied form *Lankesteria culicis* of *Aëdes argenteus*, studied by the writer (1911*a*), the gametes produced by each gregarine are alike in size, but differ from one another in that those produced by one gregarine have smaller nuclei than those produced by the other (Fig. 465). A gamete with a small nucleus unites with one which has a large one. In the case of *Stylorhynchus longicollis*, Leger, L. (1904*h*) noted that the gametes produced by one gregarine were spherical bodies, while those produced by the other were spindle-shaped structures, each provided with a flagellum. The spindle-shaped motile gametes were actually larger than the spherical ones, so that if the former are to be regarded as the male gametes, this instance affords an exception to the general rule that the male gametes are smaller than the female (Fig. 482). In the case of the gregarine *Pterocephalus nobilis*, Leger, L. and Duboscq (1903*a*) describe the gametes which are formed from one individual as small curved structures (microgametes), and those from the other as large elongate bodies (macrogametes). In this instance there is an approach to the condition which is characteristic of the coccidia. Amongst the coccidia, female gametes or macrogametes are spherical or ovoid bodies filled with food reserve material in the form of globules, while the male gametes or microgametes are minute, elongate, sickle-shaped bodies usually provided with two flagella. The microgametes, which are provided with two flagella, are composed of chromatin covered by a thin layer of cytoplasm, and in many respects resemble the spermatozoa of higher animals (Fig. 337). A similar difference in size exists between the gametes of the pigmented blood parasites of the genera *Plasmodium* and *Hæmoproteus* and the non-pigmented *Leucocytozoon* (Figs. 383 and 391). Where a special type of individual, the gametocyte, produces a number of gametes, the actual number produced by each varies considerably in different groups. Amongst the gregarines, where two gametocytes are enclosed in a gametocyst, it is evident that the chance of gametes going astray is reduced to a minimum, so that both gametocytes produce approximately the same number of gametes. In the majority of gregarines there

are many gametes (Fig. 465); there may be not more than a dozen, as in *Schizocystis* (Fig. 469), while in the case of *Ophryocystis* each gametocyte produces only a single gamete (Fig. 468).

Amongst the true coccidia or Eimeriidea, the male and female gametocytes are not associated, but develop in separate cells of the intestine or other organ. The number of gametes produced by each individual may be very unequal in number (Fig. 337). The gametocyte (macrogametocyte) which gives rise to the macrogamete becomes directly transformed into a single macrogamete, while the microgametocyte produces a large number of microgametes. The latter are provided with flagella, and swim away in search of a macrogamete, which is not itself endowed with the powers of movement. It seems evident that the production of large numbers of microgametes is correlated with the greater uncertainty of the micro- and macro-gametes coming together. In the case of the malarial parasites and allied organisms (Hæmosporidiidea), in which fertilization takes place in the stomach of a blood-sucking insect, the macrogametocyte produces a single macrogamete, while the microgametocyte gives rise to from six to ten microgametes (Fig. 391). In the coccidia belonging to the Adeleidea, the macro- and micro-gametocytes develop in actual contact with one another. The result of this close association is that, though the macrogametocyte gives rise to a single macrogamete, the microgametocyte produces only four microgametes (Fig. 338). In the hæmogregarines of the genus *Karyolysus*, in which a similar association of the gametocytes occurs, Reichenow (1921) has shown that the microgametocyte produces only two microgametes (Fig. 457). The marked difference in size between the microgametes and macrogametes in these cases is associated with the conditions under which future development will take place. The macrogamete is provided with a large amount of cytoplasm heavily loaded with food reserve material to enable it to survive and develop without nourishment in the encysted condition. As a result of this provision, as in the case of the ovum, its power of movement has been lost. The male gamete merely functions as a fertilizing agent, for which its nucleus alone is required, and for the fulfilment of which a high degree of motility is an advantage.

CONJUGATION.—In the type of syngamy which has just been described, the two gametes unite completely and their nuclei fuse. This process is known as copulation, to distinguish it from conjugation, which occurs amongst the Euciliata. In typical conjugation two individuals associate, and one of the two nuclei, which each then possesses, passes into the other individual and unites with the nucleus which has remained stationary. As pointed out above, it is possible to regard the two ciliates as each producing two gametes, the small gamete (migratory nucleus) produced by one individual uniting with the large gamete produced by

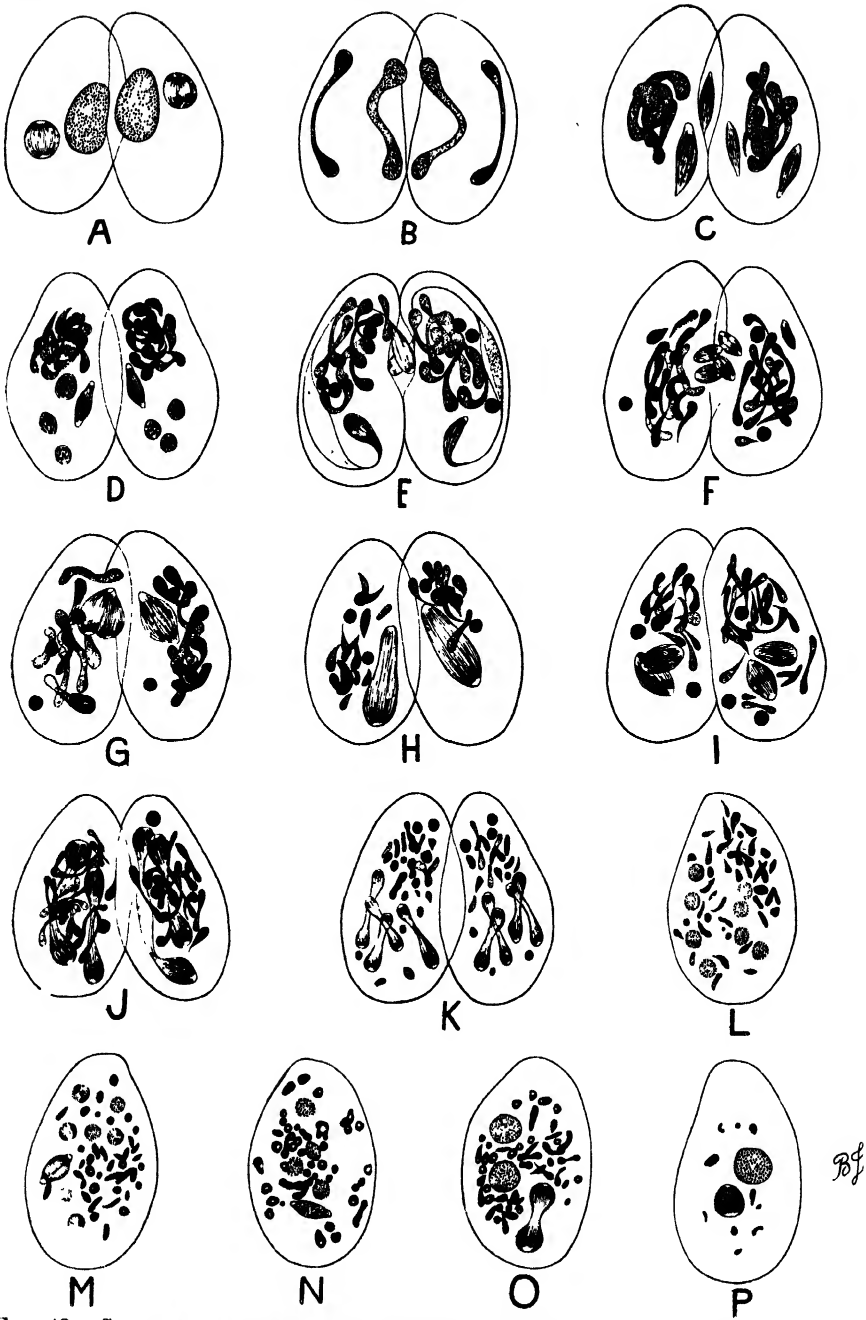


FIG. 43.—SYNGAMY IN *Paramecium putrinum*. (AFTER DOFLEIN, 1916, SLIGHTLY MODIFIED.)

[*For description see opposite page.*

the other. The process, which is an exceedingly complicated one, has been studied in detail, especially in species of *Paramecium.* In the case of *Paramecium putrinum,* for instance, each individual possesses a macronucleus and a micronucleus. When two individuals associate in conjugation, they become closely united by their peristomes and the side of their bodies behind this (Fig. 43). The macronuclei become elongated and undergo a series of divisions till a large number of fragments are produced. All these ultimately degenerate and disappear. Meanwhile, the micronuclei have divided by mitosis, and the two nuclei thus formed in each ciliate again divide by mitosis. At this stage each ciliate or *conjugant,* as it is called, contains four nuclei and a number of degenerating bodies derived from the macronucleus. Three of the nuclei in each now degenerate, so that each conjugant is left with only one. This one now divides again, and of the two resulting nuclei in each conjugant, which as far as can be seen are exactly alike, one is a stationary nucleus and the other a migratory one. The migratory nucleus of each conjugant now passes over and fuses with the stationary nucleus of the opposite conjugant. The resulting nucleus, which is a zygote nucleus, now divides to give rise to two, these two to give four, and the four to produce eight nuclei. At this stage the ciliates, each of which has eight nuclei and still the remains of the degenerating nuclei, separate from one another and swim away. Of the eight nuclei, four increase in size and become macronuclei, three degenerate, while the remaining one retains its character as a micronucleus. The latter divides to form two micronuclei, and this is followed by division of the ciliate itself in such a manner that two of the macronuclei and one of the micronuclei pass to each daughter ciliate. At the next division of these daughter ciliates the micronucleus divides, and each resulting ciliate receives one of the two macronuclei and one of the two micronuclei. Thus, the nuclear condition of the original ciliate is regained. At all subsequent divisions of the ciliate, both the macro- and micro-nuclei divide.

A. Two associated conjugants with intact macronuclei and commencing division of micronuclei.
B. Macronuclei and micronuclei dividing.
C. Divided-up macronuclei and two dividing micronuclei in each conjugant.
D. Three of the four micronuclei in each conjugant have degenerated, while the remaining one is commencing to divide.
E. The micronucleus of each conjugant is drawn out into a long spindle.
F. Four resulting micronuclei near the point of union of the two conjugants.
G. Union of the micronuclei in pairs.
H-L. Progressive division of the micronuclei till each conjugant has eight. The conjugants finally separate (L.)
M-N. Three micronuclei degenerate, four become macronuclei, while one remains and divides. The ciliate divides.
O. One product of division of the form with four macronuclei and two micronuclei. It contains two macronuclei and one dividing micronucleus. The ciliate divides.
P. One product of the division of the form with two macronuclei and two micronuclei. It contains one macronucleus and one micronucleus, and thus resembles the ciliates before they commenced conjugation.

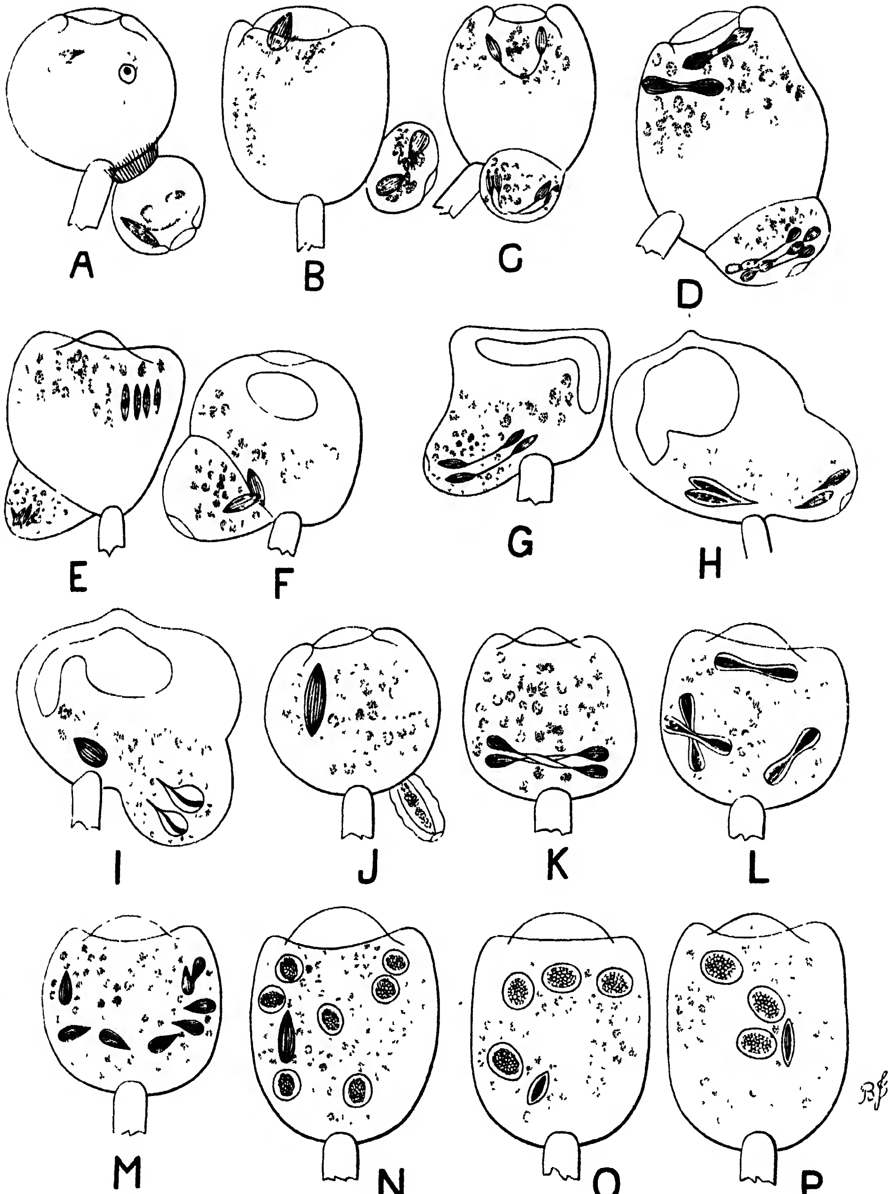


FIG. 44.—DIAGRAMMATIC REPRESENTATION OF NUCLEAR CHANGES DURING SYNGAMY IN *Vorticella nebulifera*. (AFTER MAUPAS, 1889.)

[*For description see opposite page.*

This complicated process is best comprehended by reference to the diagram (Fig. 43). Except for variations in detail, the conjugation of other ciliates in which the process has been studied takes place in a similar manner. In *Paramecium putrinum* the two conjugants are equal in size. In other ciliates a large individual conjugates with a smaller one, while the most extreme condition is reached in *Vorticella* and its allies, in which a small free-swimming ciliate budded off from a large pedunculate individual conjugates with one of the large forms (Fig. 44). The macronuclei in both degenerate, and the micronuclei undergo a number of divisions, as in *Paramecium.* All these degenerate except one which divides to give rise to a stationary and a migratory nucleus. Each individual, one a large and the other a small one, now contains two nuclei. Exchange of nuclei then occurs, as in *Paramecium*, but the small individual, instead of proceeding to further development, shrinks and dies, while the large individual alone survives. The single nucleus of the large surviving individual divides repeatedly, and a number of macronuclei and one micronucleus are produced. By successive divisions of the ciliate, similar to those occurring in *Paramecium putrinum*, the original condition is regained. In the case of *Paramecium caudatum* the process of syngamy is similar to that of *P. putrinum* (Fig. 45), but in the case of *P. aurelia*, owing to the fact that the ciliate possesses two micronuclei instead of one, it is modified in certain respects. When conjugation occurs, the two micronuclei of each conjugant divide twice, so that eight are formed. Of these seven degenerate, leaving in each conjugant one micronucleus and one degenerating macronucleus. The single micronucleus divides and exchange of nuclei occurs, as in *P. putrinum* and *P. caudatum*. After union of the two nuclei the single nucleus divides twice till four are present, and of these two become macronuclei and two remain as micronuclei. Each of the latter divides once, so that in each ciliate there are now two macronuclei and four micronuclei. The ciliate

A. Union of the small free-swimming conjugant with the large attached one.
B. Fragmentation of the macronuclei and division of the micronuclei.
C, D, E. Further divisions of the micronuclei leading to four in the large conjugant and eight in the small one.
F. All the daughter micronuclei have degenerated except one in each conjugant.
G. The two micronuclei are dividing with the axis of division across the plane of union of the two conjugants.
H. The two micronuclei in the large conjugant are uniting, while those in the small one remain separate.
I. The two micronuclei in the large conjugant have united, while those in the small one are degenerating.
J. The micronucleus of the large conjugant is dividing, while the small conjugant is shrinking.
K. The small conjugant has disappeared, while the micronuclei of the large one are dividing.
L, M. Further divisions of the micronuclei to give rise to eight.
N. Transformation of seven micronuclei into macronuclei and division of remaining micronucleus.
O, P. Division of the body has taken place, giving rise to an individual with four macronuclei and one micronucleus (O), and one with three macronuclei and one micronucleus (P). By further divisions the original condition is reached in which the micronucleus and one macronucleus are present.

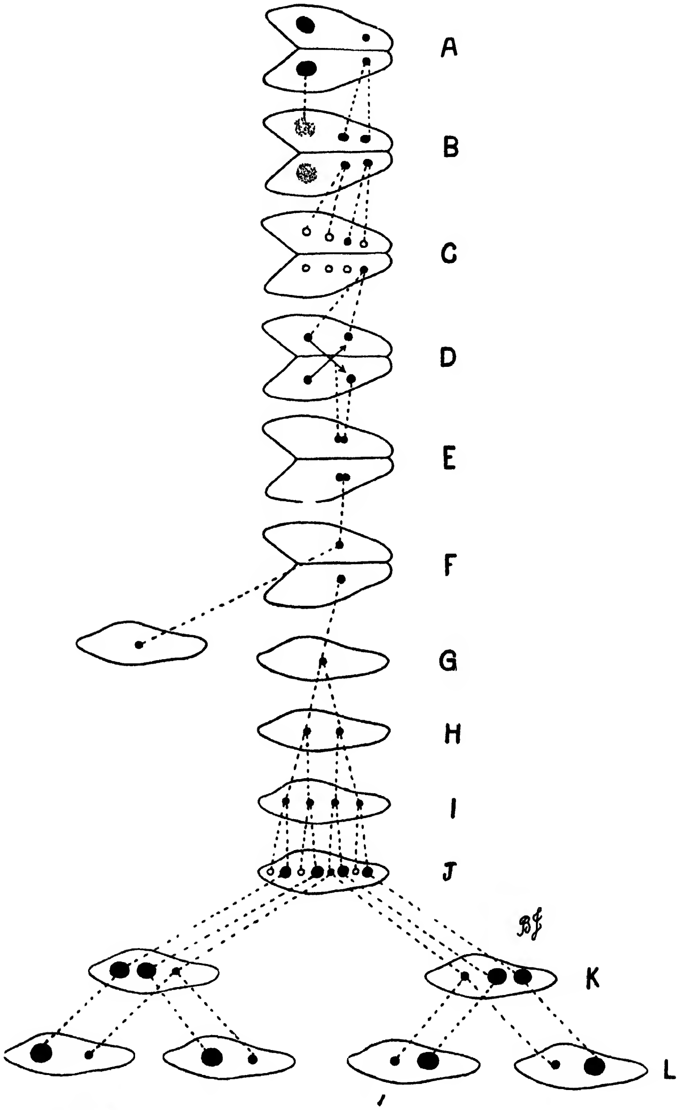


FIG. 45.—DIAGRAMMATIC REPRESENTATION OF THE NUCLEAR CHANGES DURING CONJUGATION OF *Paramecium caudatum.* (AFTER JENNINGS, 1920.)

A. Two associated conjugants.
B. Degeneration of macronucleus and first division of micronucleus.
C. Second division of micronuclei to give rise to four, of which three degenerate.
D. Division of remaining micronuclei to produce the gamete nuclei.
E-F. Union of gamete nuclei. G. Separation of the conjugants.
H-J. Division of the nuclei to give rise to eight, of which four increase in size to become macronuclei, while three degenerate.
K. After division of the single micronucleus the ciliate itself divides.
L. After a further division of the micronucleus the daughter ciliates again divide to give rise to the normal type.

which has separated from its partner divides into two daughter ciliates, each of which has a single macronucleus and two micronuclei, as in the original conjugants.

In the conjugation of *Collinia branchiarum* described below, the two ciliates unite as in *Paramecium*, and exchange of nuclei takes place (Fig. 495). The macronuclei, however, behave in a remarkable manner. Each becomes much elongated, and when exchange of micronuclei is taking place, the two long macronuclei arrange themselves side by side across the point of union of the ciliates in such a manner that half of each macronucleus is in each ciliate. When the ciliates separate the macronuclei divide, so that

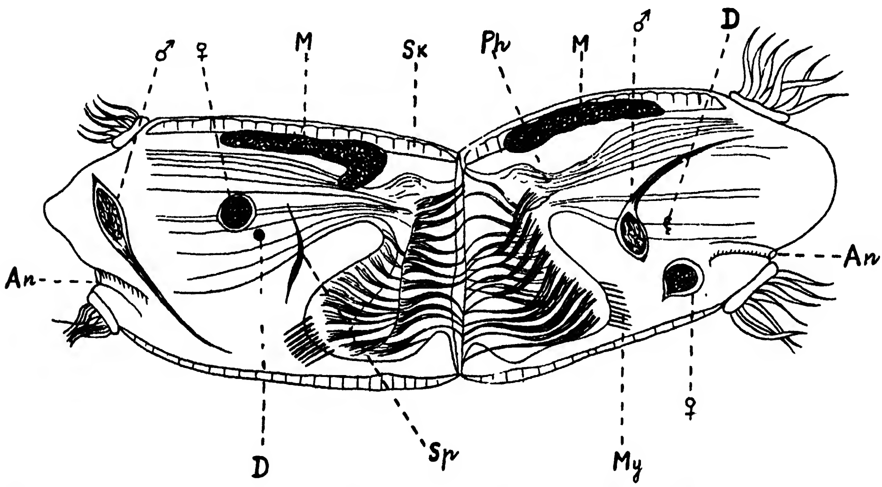


Fig. 46.—Conjugation of the Ciliate *Cycloposthium bipalmatum*, showing Differentiation of Conjugating Nuclei into Male (♂) and Female (♀) (× *ca.* 300). (After Dogiel, 1923.)

M., Macronucleus; Sk., skeletal plate; An., anus; Ph., pharynx; My., myonemes; D., degenerating micronuclei; Sp., remains of central part of spindle.

each ciliate receives half of each macronucleus. Though this occurs, the macronuclei ultimately degenerate, and a new macronucleus is formed from the micronucleus. It will thus be seen that in the Euciliata each of the two conjugants ultimately contains two nuclei which are exactly alike, except that one is a migratory or male nucleus, and the other a stationary or female nucleus. This difference in behaviour is the only indication of sex differentiation. In the case of *Cycloposthium bipalmatum*, a ciliate parasitic in the intestine of the horse, Dogiel (1923, 1925) has noted that, though conjugation between two individuals takes place in the usual manner, the two nuclei which take part in the syngamic process differ in that the migratory one assumes the characters of a male gamete in becoming a

filament provided with a head, while the stationary one retains its original form (Fig. 46). This observation is a confirmation of the view that the migratory nuclei in other ciliates are actually male nuclei.

GONOMERY.—A remarkable process of syngamy was described by Hartmann and Nägler (1908) for *Sappinia diploidea*, an amœba isolated from lizards' fæces. The amœba is peculiar in being binucleate, the two nuclei lying close together (Fig. 47). When encystment occurs, two individuals enter a common cyst. The two nuclei of each individual now fuse and then undergo reduction divisions, the reduction bodies degenerating. After this the two amœbæ unite, the nuclei approach one another, but do not fuse. The amœba then leaves the cyst and commences to

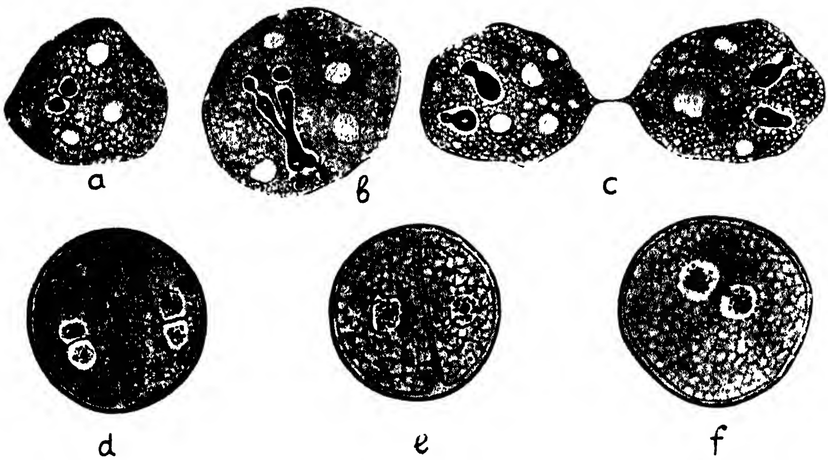


FIG. 47.—*Sappinia diploidea:* FREE AND ENCYSTED STAGES (× *ca.* 1.500). (AFTER HARTMANN AND NAGLER, 1908.)

a. Usual form with two nuclei. *b.* Form with dividing nuclei.
c. Dividing form producing two binucleated daughter amœbæ.
d. Two amœbæ in common cyst. *e.* The two nuclei in each amœba have united.
f. The bodies of the two amœbæ have fused, giving rise to a binucleated amœba which escapes from the cyst and reproduces by binary fission, as at *a*, *b*, and *c*.

multiply by binary fission, the two nuclei dividing by mitosis side by side. These nuclei are regarded as gamete nuclei, which, however, do not actually unite, though dividing many times during asexual reproduction till encystment again occurs. This condition is one of delayed union of gamete nuclei, a process which is known to occur in higher animals, and which has been termed *gonomery*.

METHOD OF UNION OF GAMETES.—The actual union of gametes during syngamy takes place in a variety of ways, which are dependent on the structure of the gametes themselves. In the case of *Polytoma uvella*, *Copromonas subtilis*, and other forms, the two flagellates approach one

another, and unite first by their anterior ends near the flagellar origin (Figs. 42 and 48). During this process the flagellates are actively motile. Their nuclei approach one another and come into contact, and the nuclear membrane disappears at the line of contact till a common membrane is formed. In the case of *Cercomonas longicauda,* Woodcock (1916) observed union to take place first near the posterior end (Fig. 41).

Union of gametes within the gametocysts of gregarines takes place in a similar manner. As already explained, sometimes the gametes are

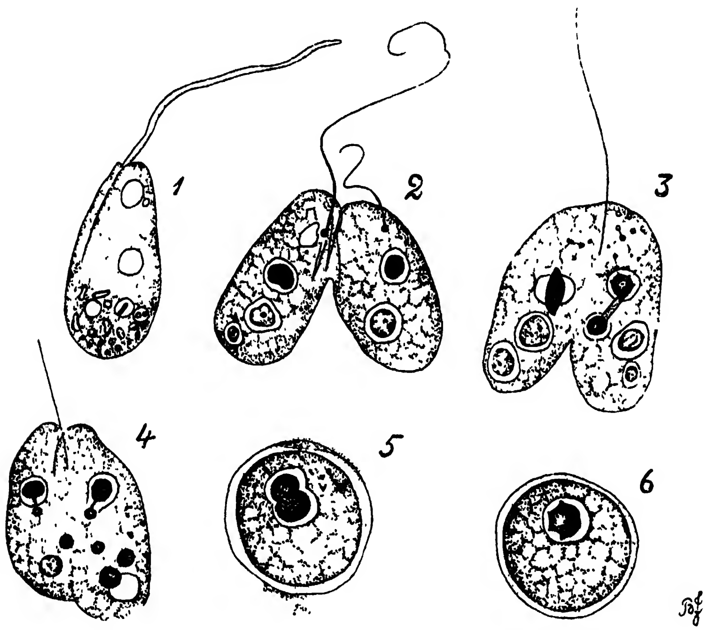


FIG. 48.—SYNGAMY OF *Copromonas subtilis* (× *ca.* 2,000). (AFTER DOBELL, 1908.)

1. Individual flagellate as seen in living condition.
2. Early stage in union of gametes.
3. Nuclei dividing to form first reduction body.
4. Nuclei dividing to form second reduction body.
5. Union of nuclei and formation of cyst
6. Fully formed zygote in cyst.

alike, and are merely spherical bodies which, coming into contact with one another, gradually fuse, while their nuclei unite. In other cases the gametes produced by one gregarine are elongate and provided with flagella, as in *Stylorhynchus*, while those produced by the other are spherical bodies (Fig. 482). Union takes place by one of the elongate flagellated gametes attaching itself to one of the spherical forms by its pointed anterior extremity, after which fusion takes place, while the flagellum disappears. Amongst the coccidia the minute flagellated microgamete swims actively and comes in contact with one of the larger immobile

macrogametes. Sometimes this occurs before the oöcyst has formed; at other times after its formation, in which case a pore, the micropyle, is present at one end of the cyst, and through it the microgamete makes its way. The microgamete enters the cytoplasm of the macrogamete, which immediately commences to secrete a substance which closes the micropyle. Though several microgametes may be attracted towards one macrogamete, immediately one has entered its cytoplasm this attraction ceases. The nucleus of the macrogamete has meanwhile been drawn out into a long spindle, the *fertilization spindle*, on the fibres of which the chromatin granules are distributed. The microgamete nucleus breaks up into granules, which gradually become distributed upon the fertilization spindle. The spindle now retracts, and a spherical nucleus containing chromatin from both the macrogamete and microgamete is again formed (Fig. 337).

Amongst the pigmented blood parasites of the genera *Plasmodium* and *Hæmoproteus* a similar type of union occurs. The nucleus of the macrogamete moves towards the surface of the body, which is raised up at this point into a small elevation. The elongate motile microgamete enters this elevation, and its nucleus unites with that of the macrogamete (Figs. 383 and 391).

Amongst the Euciliata, when conjugation occurs amongst free-swimming forms, it is usually by the peristomes that they become attached to one another. Actual continuity of cytoplasm appears to take place just behind the peristomes, to allow of the interchange of nuclei, as described above. In the attached forms, such as *Vorticella*, conjugation, as already noted, takes place between a large attached individual and a small free-swimming ciliated form which has been budded off from another individual. The small free-swimming form attaches itself to the larger one at a point near the insertion of its stalk, and when exchange of gamete nuclei has occurred it degenerates (Fig. 44).

METHOD OF FORMATION OF GAMETES.—The actual method by which gametes are formed from gametocytes varies to some extent. Amongst the gregarines, the nucleus of the gametocyte multiplies by a series of divisions till the requisite number of nuclei are present (Fig. 465). These are then arranged upon the surface of the gametocyte, and little elevations of the cytoplasm are formed. Into each of these there passes a nucleus. Each small cytoplasmic elevation or bud, which has acquired the characteristic form of the gamete, is now separated by a constriction. A large amount of the cytoplasm is usually left over as a *residual body*. In the case of the coccidia and allied forms, where there is an extreme condition of anisogamy, one gametocyte, the macrogametocyte, gives rise to a single macrogamete. It is supposed that this transformation takes

place by the extrusion of one or more reduction bodies. In the case of the microgametocyte, nuclear multiplication takes place till numbers of nuclei are formed (Fig. 337). These nuclei at first appear as minute aggregations of chromatin granules. They change their form on the surface of the cytoplasm till they appear as dense comma-shaped structures. Each is then separated with a small amount of cytoplasm, which contributes to the formation of flagella. In the blood parasites belonging to the genera *Plasmodium*, *Hœmoproteus*, and *Leucocytozoon*, the macrogametocyte produces a single macrogamete, as in the coccidia, by the rapid extrusion of reduction bodies. The microgametocyte gives rise in the course of a few minutes to six or ten microgametes by a violent process known as *exflagellation*, which occurs normally in the stomach of the invertebrate host, but which may be observed in an ordinary moist preparation of blood under the microscope (Fig. 381). The details of the process will be described below in the section devoted to these parasites, but it may be noted here that the function of the reduction bodies referred to above is far from clear, and the assumption that the process is comparable with the formation of polar bodies during maturation of the ovum of higher animals does not appear to be correct.

AUTOGAMY.—A process of syngamy which may be defined as self-fertilization has been described for certain Protozoa under the name of autogamy. In its most complete form the nucleus of a single individual divides to form two daughter nuclei. Each of these undergoes reduction divisions, after which the two surviving nuclei unite. In the case of *Entamœba coli*, Schaudinn (1903) described autogamy in the encysted stages. The single nucleus of the encysted form divides to give rise to two nuclei. Each of these gives off two reduction bodies, after which they divide to form four nuclei, which are arranged in pairs at opposite sides of the cyst. One of each pair is a stationary nucleus and one a migratory nucleus. The migratory nuclei move to opposite sides of the cyst, where they unite with the stationary nuclei. The cyst again has two nuclei, which proceed to divide till the characteristic eight nuclear stage is reached. The writer (1907) saw certain stages in the development of the cysts of *Entamœba muris*, which appeared to supply a confirmation of Schaudinn's account of *E. coli*, but there is little doubt that the appearances were capable of another interpretation. All evidence goes to show that no such process actually occurs in the cysts of *E. coli* or any other amœba. A somewhat similar process was described by Prowazek (1904*a*) in the cysts of *Prowazekella lacertœ*, while Schilling (1910) recorded its occurrence in *Trypanosoma lewisi*. It seems perfectly clear that in none of these cases was there sufficient evidence to justify the conclusions which were made.

Hartmann (1909) gave a general account of autogamy amongst Protista, but a perusal of his paper shows that most, if not all, of the alleged instances are based on very slender evidence.

PEDOGAMY.—There is another type of self-fertilization which differs from true autogamy in that a single individual first divides into two daughter forms after division of its nucleus. When the nucleus of each has undergone maturation or reduction divisions, the two daughter cells which are gametes unite. The process which is known as *pedogamy* has

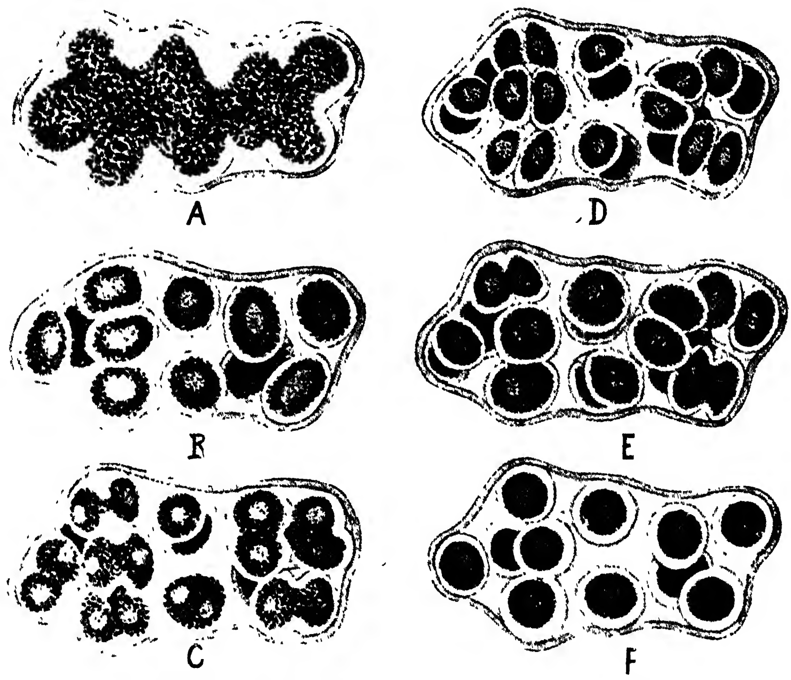


FIG. 49.—PEDOGAMY IN *Actinosphærium eichhorni* (× 80). (FROM LANG, 1901, AFTER RICHARD HERTWIG, 1898.)

A. A single multinucleated individual in a primary cyst.
B. Division into a number of uninucleated individuals which become enclosed in secondary cysts.
C. The contents of each secondary cyst divide into two.
D. The division completed, after which each nucleus undergoes two reduction divisions.
E The two gametes in each secondary cyst unite.
F. Secondary cysts containing zygotes resulting from the union of the gametes.

been studied by Richard Hertwig (1898) and others in the multinucleated Heliozoon *Actinosphærium eichhorni* (Fig. 49). An individual encysts and divides into a number of uninucleate forms, which become enclosed in secondary cysts. Within each secondary cyst a further division into two individuals takes place. The nucleus of each of these undergoes two reducing divisions, after which union takes place. In this case the

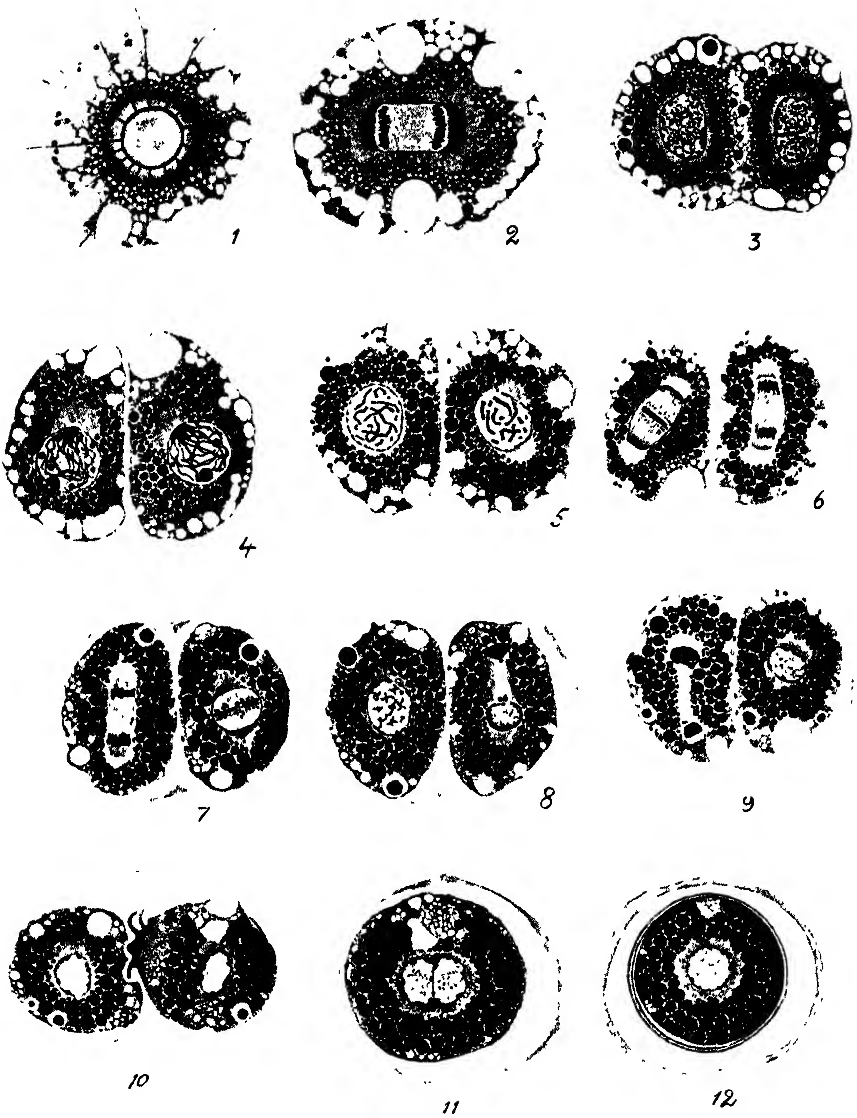


FIG. 50.—PEDOGAMY IN *Actinophrys sol*, EHRENBERG (× *ca.* 800). (AFTER BĚLAŘ, 1923.)

1. Loss of pseudopodia and their axial filaments.
2. First nuclear division and commencing formation of gelatinous envelope.
3. Two gamete nuclei in early division stage in the dividing cytoplasm within the cyst membrane.
4. Two separate gametes each with a nucleus in process of first maturation division (reduction division).
5. Later stage of reduction division of the gamete nuclei: the chromosomes in conjugation.
6. Two later stages in the reduction division of the gamete nuclei.
7. Completion of the reduction division: each gamete has a nucleus and a reduction body (degenerate nucleus).

[*Continued on p.* 88.

two gametes are formed from a single individual in the secondary cyst. In such an example there is an extreme instance of inbreeding. More recently Bělař (1921*b*, 1923) has described in detail a similar process of pedogamy for another Heliozoon, *Actinophrys sol* (Fig. 50). A single uninucleate individual encysts and divides into two daughter forms, which become gametes. The nuclei undergo two divisions, one of which is a reducing division in that the number of chromosomes is halved. One of the products of each nuclear division degenerates. The two gametes within the cyst then unite. The development is comparable with that which occurs in the secondary cysts of *Actinosphærium*. In the case of *Actinophrys sol*, Schaudinn (1896*a*) stated that two individuals entered the cyst, but doubt was thrown upon this by Distaso (1908) and Prowazek (1913*b*), who stated that the two gametes were derived from one individual. Bělař has finally confirmed the statements of the latter observers. He has also noted that occasionally two individuals encyst together, and that each divides to form two gametes, so that four gametes occur within the cyst. After the maturation divisions have taken place, the gametes unite in such a way that those formed from one individual unite with those from the other. In some cases, of the two gametes formed from one individual, one is motile and the other not, so that a distinction between male and female gametes can be drawn (Fig 50, 10).

PARTHENOGENESIS.—Amongst higher animals it sometimes happens that the ovum, which usually develops only after fertilization, does so without this having taken place. It is evident from what has already been explained that in such a case the nucleus will only possess half the number of chromosomes that it would have had if fertilization had occurred. It has been found that during the parthenogenetic development of the ovum the nucleus behaves in a variety of ways, by which the double number of chromosomes is regained. Another feature of parthenogenesis is that, though the ovum which develops without fertilization may give rise to the same type of individual as it does when fertilized, this is not necessarily the case. Thus, the ova of the honey-bee if fertilized develop into females, if unfertilized into males. Amongst the Protozoa, several observers have attempted to establish the occurrence of parthenogenesis. The most notable instance is that described by Schaudinn (1902*a*) for the malarial parasite, *Plasmodium vivax* of man. This observer supposed that the female macrogamete, which usually develops only after fertiliza-

8. Two stages in second maturation division of the gamete nuclei.
9. Completion of second maturation division and formation of second reduction body: the two reduction bodies are still present in later gamete.
10. Mature gametes, showing sexual dimorphism: the male gamete has pseudopodia.
11. Union of two gametes and commencing fusion of their nuclei.
12. Zygote within its cyst.

tion in the mosquito's stomach, is sometimes able to do so in the human blood-stream without fertilization. The nucleus is described as dividing into two parts, one of which is cast off with a portion of cytoplasm and degenerates. The remaining nucleus multiplies, and reproduction by schizogony occurs. In this manner it is supposed that the asexual or schizogony cycle is started again, and it was claimed that this afforded an explanation of the occurrence of relapses in malaria. The writer has long held and taught that the parthenogenetic forms depicted by Schaudinn were instances of red blood-corpuscles doubly infected with a gametocyte and a schizont (Plate XII, 19, p. 926). Thomson, J. D. (1917), also came to this conclusion, and showed conclusively that Schaudinn's figures purporting to represent a parthenogenetic process were really instances of doubly infected cells.

The cases of parthenogenesis recorded by Prowazek (1904) for *Herpetomonas muscarum* and by Gonder (1910*a*, 1911*b*) for *Theileria parva* have even less evidence to support them than the instance described above.

The various methods by which syngamy is accomplished amongst the Protozoa may be grouped as follows:

1. *Copulation.*—Complete union of two individuals.
   - (1) Two individuals having the characters of the ordinary reproducing forms unite.
     - (*a*) The uniting forms are equal in size (isogamy).
     - (*b*) The uniting forms are unequal in size (anisogamy).
   - (2) Two individuals (gametocytes) give rise to a number of smaller forms (gametes) which unite in pairs.
     - (*a*) The gametes produced by the gametocytes are equal in size and characters (isogamy).
     - (*b*) The gametes produced by one individual are unlike those produced by the other (anisogamy).
       - (i.) The number of gametes produced by the gametocytes are equal, or approximately equal, in number.
       - (ii.) One gametocyte (macrogametocyte) gives rise to one large gamete (macrogamete), while the other (microgametocyte) gives rise to a variable number of small motile gametes (microgametes).

2. *Conjugation.*—Two individuals (conjugants) associate, their nuclei divide, and exchange of daughter nuclei takes place, after which the conjugants separate.
   - (1) The conjugants are equal in size.
   - (2) The conjugants are unequal in size, one, a small one (microconjugant), associating with a large one (macroconjugant). In some cases, after interchange of nuclei the microconjugant degenerates.

3. *Autogamy.*—The nucleus of a single individual divides into two. Each of these daughter nuclei undergoes reduction divisions, after which they unite. It is extremely doubtful if this process ever occurs.

4. *Pedogamy.*—A single individual divides into two. Reduction divisions of the nuclei of these two daughter individuals which are gametes take place, after which the gametes unite and their nuclei fuse.

5. *Parthenogenesis.*—Part of the nucleus of a gamete, which normally develops only after union with another gamete, is extruded, after which multiplication occurs. There appear to be no convincing records of such a process amongst the Protozoa.

NUCLEAR DIVISION AMONGST THE PROTOZOA.

The division of a nucleus which takes place by simple constriction into two parts without formation of chromosomes is known as *amitotic division*, to distinguish it from *mitotic division*, in which definite chromosomes and a spindle, associated with the presence of centrosomes, occur as described above for the nuclear divisions of the cells of higher animals. Between what appears to be true amitosis and mitosis there occur many gradations. In some cases the appearances are in every way comparable with what has been described above as typical mitosis in the cells of higher animals. In other instances the nuclear membrane persists, and the whole process of mitosis occurs within the nuclear membrane. In other cases, again, there appear to be no centrosomes associated with mitosis within the nuclear membrane, though many observers describe an intranuclear structure, called the *centriole*, which is supposed to function as a centrosome. As regards the nature of this body and its actual existence there is much difference of opinion. That the formation of a spindle may occur without definite centrosomes being identifiable has long been recognized in higher plants, so there is no reason to suppose that this may not happen amongst the Protozoa. When mitosis occurs within the nuclear membrane, definite chromosomes may be formed at the equator of the spindle, and these divide into daughter chromosomes in the usual manner. In other cases, though a spindle is formed, the chromatin granules become arranged irregularly upon the spindle fibres without uniting into definite chromosomes. No equatorial plate is formed, and the nucleus merely constricts into two parts. It is possible that in some of these instances of irregularly arranged chromatin granules there are produced a very large number of minute chromosomes which actually divide. In order to distinguish these intermediate types of mitosis from typical mytosis, the term *promitosis* has been introduced by Nägler (1909).

A good illustration of complete mitosis is afforded by the nuclear

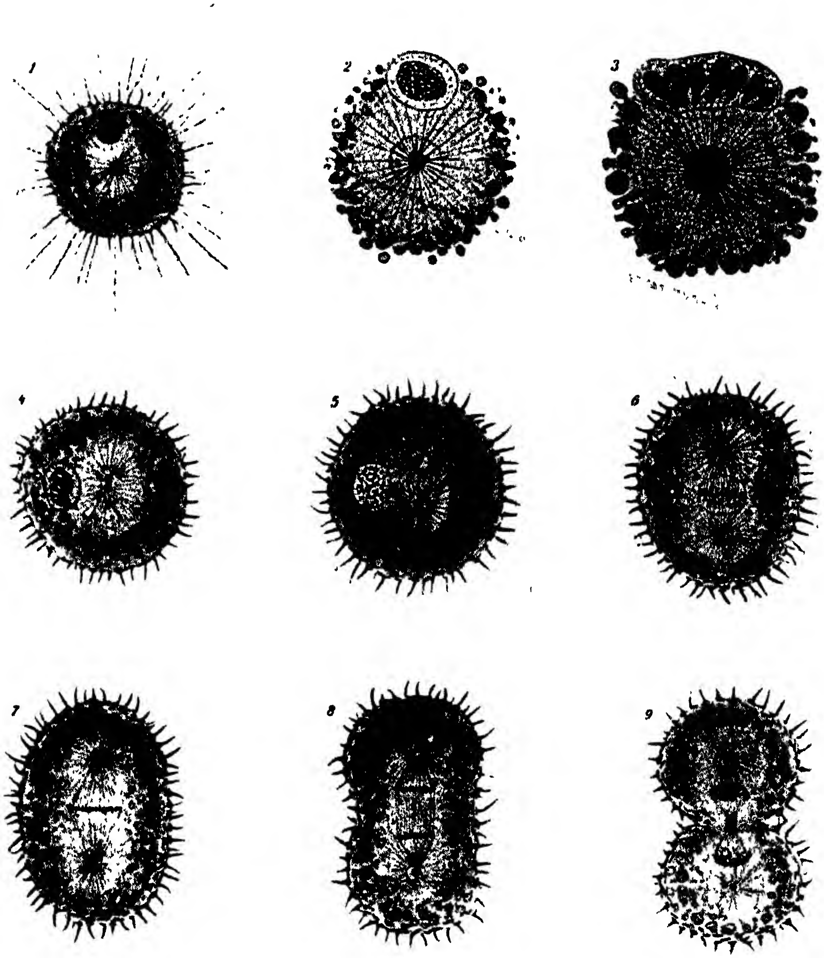


FIG. 51.—STAGES IN THE NUCLEAR DIVISION OF *Acanthocystis aculeata* IN WHICH THE CENTRAL GRANULE FUNCTIONS AS A CENTROSOME (× *ca.* 600). (AFTER SCHAUDINN, 1896.)

1. Ordinary individual, showing nucleus and central granule, from which radiate the axial fibres of the pseudopodia (axopodia).
2, 3. Changes in nucleus at commencement of division.
4. Division of central granule and nucleus in spireme stage.
5, 6. Formation of chromosomes in nucleus, as it takes up a central position on the spindle which forms between the two granules.
7. Disappearance of nuclear membrane: formation of equatorial plate.
8. Separation of daughter plates of chromosomes.
9. Cytoplasm dividing and two nuclei and central granules returning to the condition of individual at 1.

division of *Acanthocystis aculeata*, one of the Heliozoa, as described by Schaudinn (1896*b*), (Fig. 51). In the ordinary individual the centre of the body is occupied by a granule, from which radiate the axial fibres supporting the fine pseudopodia. The nucleus, which has a membrane and large central karyosome, lies at one side of the central granule. When division is to take place, the nucleus increases in size and the karyosome becomes loculated, broken into a number of separate parts, and finally disintegrated as minute granules which arrange themselves in the form of a spireme or coiled thread. Meanwhile the supporting fibres of the pseudopodia have disappeared, while radiating fibres develop in the cytoplasm in connection with the central granule, which, on account of the part it plays in nuclear division, must be regarded as the centrosome. The latter structure divides, and as the two daughter centrosomes separate a spindle is formed between them, while radiating fibres form two asters. The nucleus, within which the spireme has segmented into a number of separate parts, now moves to the equator of the spindle. The nuclear membrane disappears, and a number of small chromosomes take up a position on the spindle as an equatorial plate. The individual chromosomes divide, and there are formed two daughter plates which move towards opposite poles of the spindle. At this stage the body of the Heliozoon, which has become elongated, begins to show a constriction around its centre. The spindle is finally divided at its centre, and the daughter chromosomes of each plate become transformed into a karyosome, while a new nuclear membrane is developed. The centrosome remains as the central granule of the daughter individual which has been formed, and new axial fibres are developed. In this division, practically all the stages of mitosis as seen in the Metazoan cell occur.

Typical examples of mitosis occur also in the case of gregarines, the nuclei of which divide repeatedly to form the gamete nuclei. Muslow (1911) has described the process as it occurs in *Monocystis rostrata*, one of the species of *Monocystis* which inhabit the vesicula seminalis of the earth-worm. The resting nucleus consists of a nuclear membrane and large central karyosome. When the first nuclear division is to take place after two gregarines have become encysted together in the gametocyst, the large karyosome breaks up, while a long twisted thread of chromatin granules appears at one side of the nucleus (spireme stage). Meanwhile, from two small areas on the surface of the nuclear membrane, radiations appear in the cytoplasm to form the two asters. Between these, spindle fibres develop, and with the disappearance of the nuclear membrane the chromatin thread becomes segmented into eight looped chromosomes, which arrange themselves at the equator of the spindle. Each chromosome becomes divided longitudinally, and the two groups of eight daughter

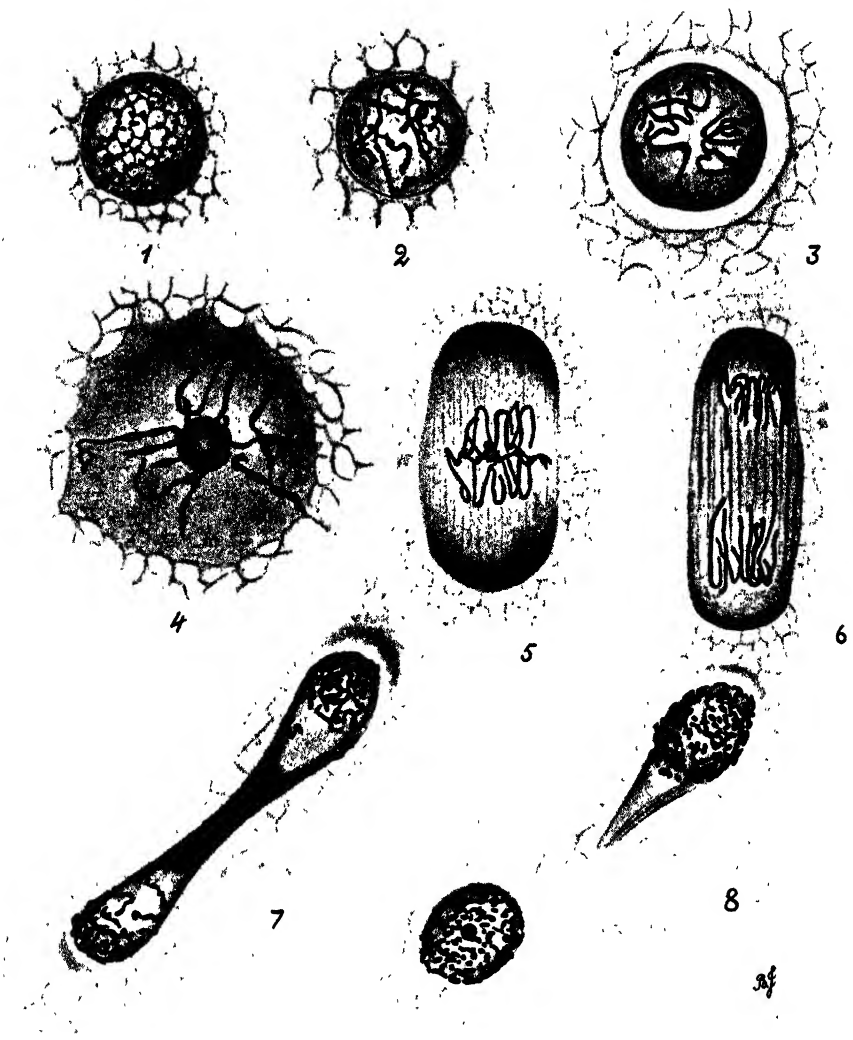


FIG. 52.—VARIOUS STAGES IN THE LATE MITOTIC DIVISION OF THE NUCLEUS OF *Monocystis rostrata* (1–3 × 2,000; 4–8 × 1,700). (AFTER MUSLOW, 1911.)

1. Resting nucleus. 2, 3. Formation of eight chromosomes.
4. Commencing splitting of the chromosomes.
5. Daughter chromosomes separating at equator of spindle, which is devoid of centrosomes and asters.
6. Daughter chromosomes moving towards the poles of the spindle.
7. Chromosomes breaking up into gametes. 8. Reconstitution of the nuclei.

chromosomes move to opposite poles of the spindle. The central part of the spindle disappears, the chromosomes break up into granules, and with the formation of a nuclear membrane the nucleus is reconstructed. In subsequent divisions the process is very similar, except that a spindle is formed without definite centrosomes or asters (Fig. 52).

Very similar mitotic divisions of the nucleus were described by Brasil (1905) also in the case of a species of *Monocystis* of the earth-worm (Fig. 53). In both these instances the nuclear membrane disappears during division, but in other cases the nuclear membrane persists during the whole mitotic division of the nucleus.

In the case of *Actinosphærium eichhorni*, the life-history of which has been described in detail by Richard Hertwig (1898) in a classic memoir,

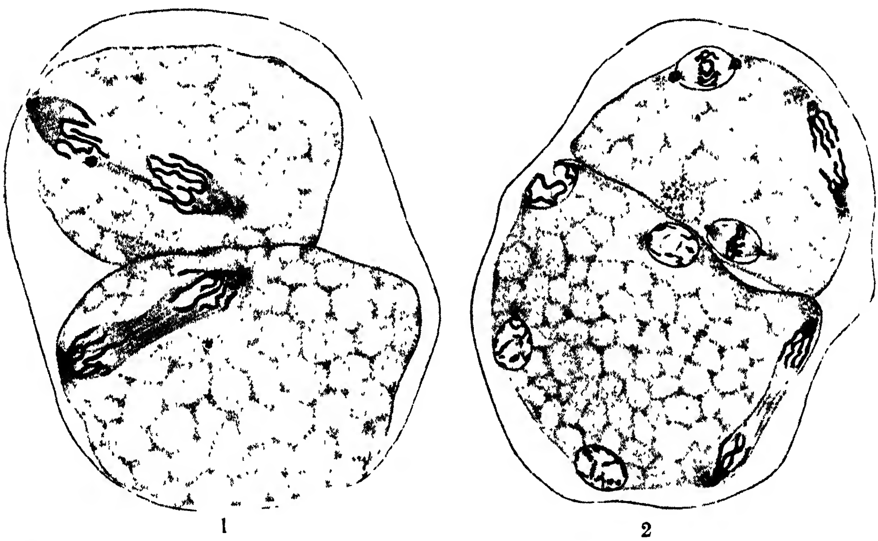


FIG. 53.—NUCLEAR DIVISIONS IN ASSOCIATED MONOCYSTID GREGARINES (*Monocystis* SP.) OF THE EARTH-WORM (× 900). (AFTER BRASIL, 1905.)

1. First nuclear division, showing centrosomes, spindles, and elongate daughter chromosomes.
2. Later nuclear divisions in various stages of mitosis.

very clear examples of mitosis occur. The multinucleate organism, as mentioned above, becomes encysted in a large primary cyst, within which it divides into a number of daughter individuals round which secondary cysts are formed (Fig. 49). Within the secondary cyst a further division into two individuals takes place. The nucleus of each of these divides by mitosis to form two nuclei, one of which degenerates. A second division of the surviving nucleus takes place, and again one of the resulting nuclei degenerates. After this, the two individuals or gametes in the secondary cyst unite and their nuclei fuse. The various nuclear

divisions take place by mitosis. When the nucleus of a gamete in the secondary cyst is about to divide for the first reduction division, there appears at one side of the nucleus an area of clear cytoplasm towards which the linin network of the nucleus with its chromatin granules is drawn (Fig. 54). Into this clear cytoplasm some of the chromatin granules of the nucleus are attracted, and by their aggregation give rise to the centrosome. It is possible the centrosome was already present, either in the nucleus or outside it, and that the commencement of its activities results in the concentration of the nuclear elements at this pole of the nucleus, and even the escape of some of the chromatin into the cytoplasm. Whether Hertwig's account of the origin of the centrosome is correct or not, when it becomes apparent it is situated at some distance from the nuclear membrane, and is surrounded by radiations, the bulk of which are directed towards the nuclear membrane. Division of the centrosome takes place, and one of the resulting pair takes up a position at the opposite pole of the nucleus. There are now two asters between which spindle fibres appear. The nucleus occupies a position between the two centrosomes, and the spindle fibres extend through the nuclear membrane and the substance of the nucleus, so that there is both an extranuclear and an intranuclear portion of the spindle. The chromatin granules of the nucleus now form a series of chromosomes which become arranged in the form of a plate across the equator of the spindle within the nuclear membrane. Each chromosome divides, and there result two daughter plates which, just behind the ends of the elongating nuclear membrane, move towards the centrosomes. The nuclear membrane, which has divided, now closes round the chromosomes, which gradually disintegrate, so that daughter nuclei are formed. One of the nuclei now degenerates. As already remarked, the nuclei of the two individuals in the secondary cysts undergo two such divisions, the description just given applying to the first of these. The second division is of a similar type, and again one of the daughter nuclei degenerates. Richard Hertwig regards both these divisions as reduction divisions, though he believed that the chromosomes actually divide in each instance. It seems reasonable to suppose, from what is now known to occur in other Protozoa, that in one of the two divisions splitting of the chromosomes does not take place, but that they separate into two groups, so that the number of chromosomes in the daughter or final gamete nucleus is halved. This is all the more probable since, in *Actinophrys sol*, an allied form which has a similar syngamic process, Bělař (1921*b*, 1923) has noted that in the first of the divisions the chromosome number of forty-four is reduced to twenty-two (Fig. 50). To return to *Actinosphærium eichhorni*, the many nuclei which an adult contains become the nuclei of the daughter individuals which form the

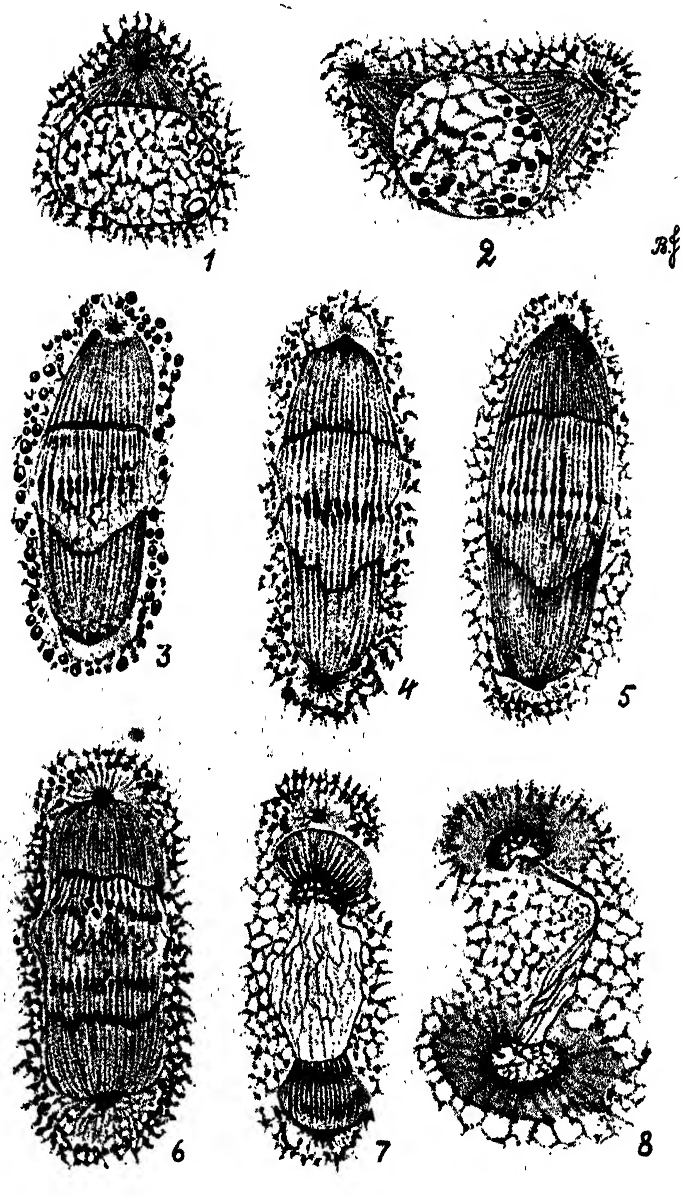
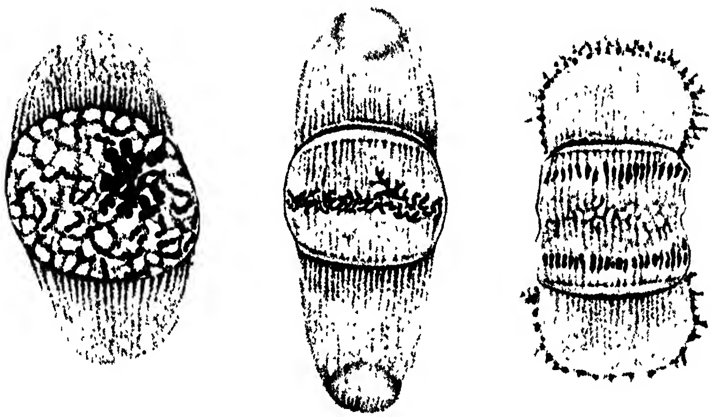


Fig. 54.—First Reduction Division of the Nucleus of One of the Two Gametes in the Secondary Cyst of *Actinosphærium eichhorni* (× *ca.* 1,200). (After R. Hertwig, 1898.)

[*For description see opposite page.*

secondary cysts. These nuclei are the result of repeated mitotic divisions of the nucleus of the parent. These divisions differ from those described as taking place in the gametes in the secondary cysts, in that definite centrosomes do not occur. Similarly, when the daughter individual in the secondary cyst divides to form the two gametes, its nucleus divides without the formation of centrosomes (Fig. 55). Indications of longitudinally arranged fibres can, however, be detected within the nuclear membrane, and also in a cone-shaped portion of cytoplasm which occupies



1 2 3

FIG. 55.—FIRST NUCLEAR DIVISION IN THE SECONDARY CYST OF *Actinosphærium eichhorni* (× *ca.* 1,200). (AFTER R. HERTWIG, 1893.)

1. Chromosomes forming in the nucleus. 2. Chromosomes arranged as an equatorial plate.
3. Daughter chromosomes separating as two plates.
No definite centrosomes appear at any stage.

the poles of the elongating nucleus. Chromosomes are formed, become arranged as an equatorial plate, and divide into daughter chromosomes in the usual manner.

As an illustration of another type of mitosis in which a definite spindle and chromosomes are formed associated with disappearance of the nuclear membrane and complete absence of centrosomes, *Amœba glebæ* (*Hartmannella glebæ*), a soil amœba described by Dobell (1914*a*), may be con-

1. Centrosome with radiations.
2. Two centrosomes at opposite poles of nucleus in which chromosomes are commencing to form.
3. The spindle has formed between the centrosomes, and chromosomes have taken up a position as an equatorial plate. 4. Commencing division of the chromosomes.
5. The chromosomes have divided and two equatorial plates are formed.
6. Passage of the daughter chromosomes towards the centrosomes.
7. Later stage, in which the nuclear membrane is closing round the chromatin granules.
8. Two daughter nuclei have formed, though the remains of the spindle and the radiations from the centrosome, which has itself disappeared, are still to be distinguished.

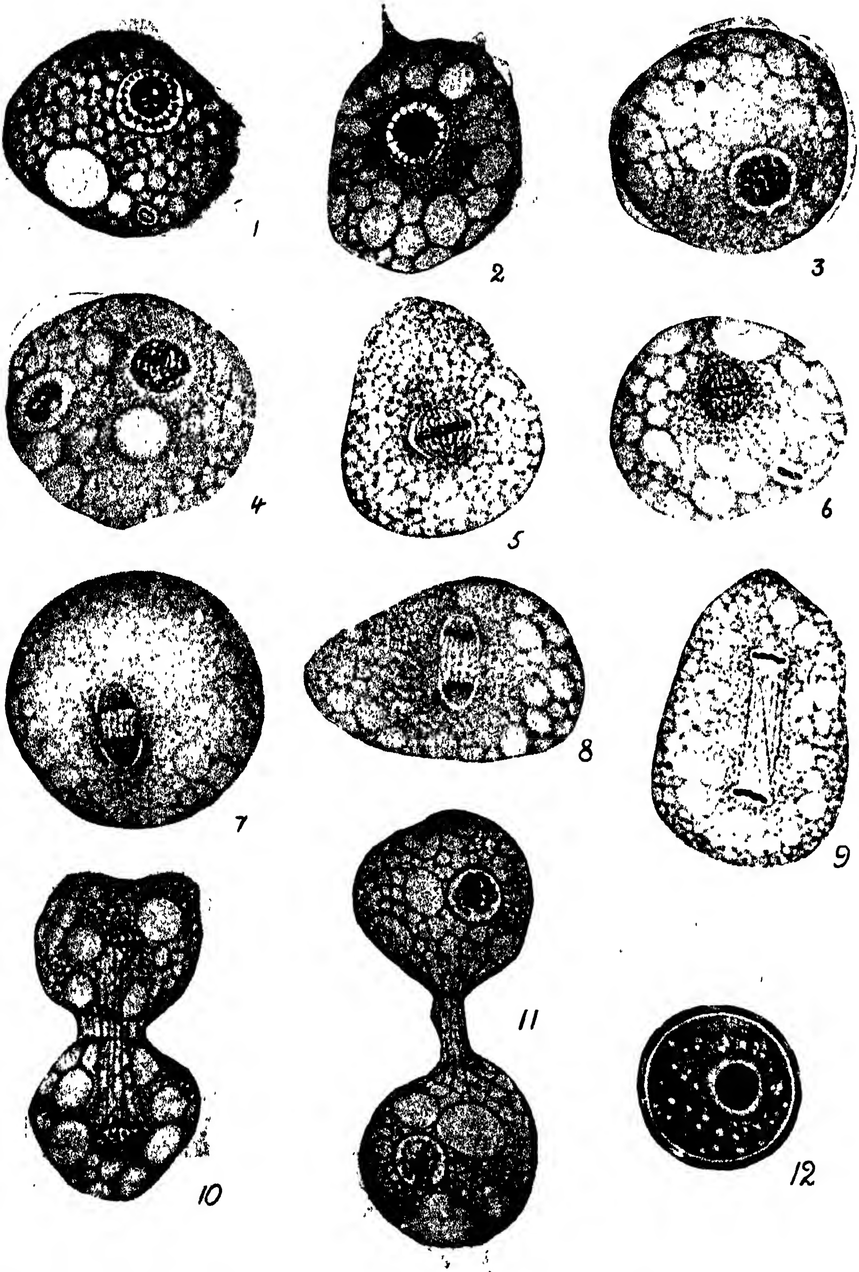


FIG. 56.—*Hartmannella glebæ:* BINARY FISSION TO SHOW VARIOUS PHASES OF NUCLEAR DIVISION (×2,000). (AFTER DOBELL, 1914.)

[For description see opposite page]

sidered (Fig. 56). The resting nucleus consists of a fairly thick membrane and a large central karyosome round which are arranged a series of granules. When nuclear division, preparatory to division of the amœba, commences, the nuclear membrane becomes thin and the karyosome fragments into a number of fine granules, while those which surround the karyosome disappear. Those originating from the karyosome run together to form larger granules, which become arranged as a long-coiled chain of beads which, decreasing in length, finally occupies the equator of the nucleus as a ring. The linin network of the nucleus now shows indications of spindle-fibre formation and the nuclear membrane disappears. The spindle, which has rounded ends and no centrosomes or asters, becomes slightly elongated, while the chromosomes, sixteen in number, which are arranged as a ring round the equator of the spindle, divide so that two rings of daughter chromosomes are formed. These separate from one another as the spindle itself becomes greatly drawn out. Finally, each ring of daughter chromosomes which has moved to the end of the spindle is broken up and a nuclear membrane is formed. The daughter nucleus is at first flattened, but gradually increases in size, and, with reconstruction of the karyosome, assumes the characters of the original parent nucleus. Before this stage is reached the amœba, which has become elongated, is divided by constriction into two parts. In this division there are no granules which could be interpreted as centrioles at the apices of the spindle, nor was it possible to discover any indications of a centrodesmose, so that it would appear that centrosomes and centrioles are completely absent.

The division of the nucleus of *Entamœba histolytica*, as seen in the encysted forms, is of a similar type, but the nuclear membrane remains throughout the process (Fig. 57). The earliest stage appears to be the division of the minute central karyosome. The two daughter karyosomes separate, while a spindle forms between them. On the equator of the spindle, which is surrounded by the elongating nuclear membrane, appear a ring of chromosomes in an equatorial plate. These divide to form daughter chromosomes, which pass towards the poles of the elongating spindle in an irregular manner. According to Kofoid and Swezy (1924*a*, 1925) the chromosome number is six. As the spindle elongates the daughter

1. Usual type of amœba · nucleus with large central karyosome surrounded by granules.
2, 3. Karyosome breaking up into granules.
4. Chromatin arranged as irregular loop.
5. Disappearance of nuclear membrane: spindle with equatorial plate of chromosomes.
6-9. Division of chromosomes to form daughter plates, which pass to the poles of the elongating spindle.
10. Commencing division of amœba.
11. Disappearance of spindle, reconstruction of nuclear membrane, and commencing reconstruction of karyosome.
12. Encysted amœba.

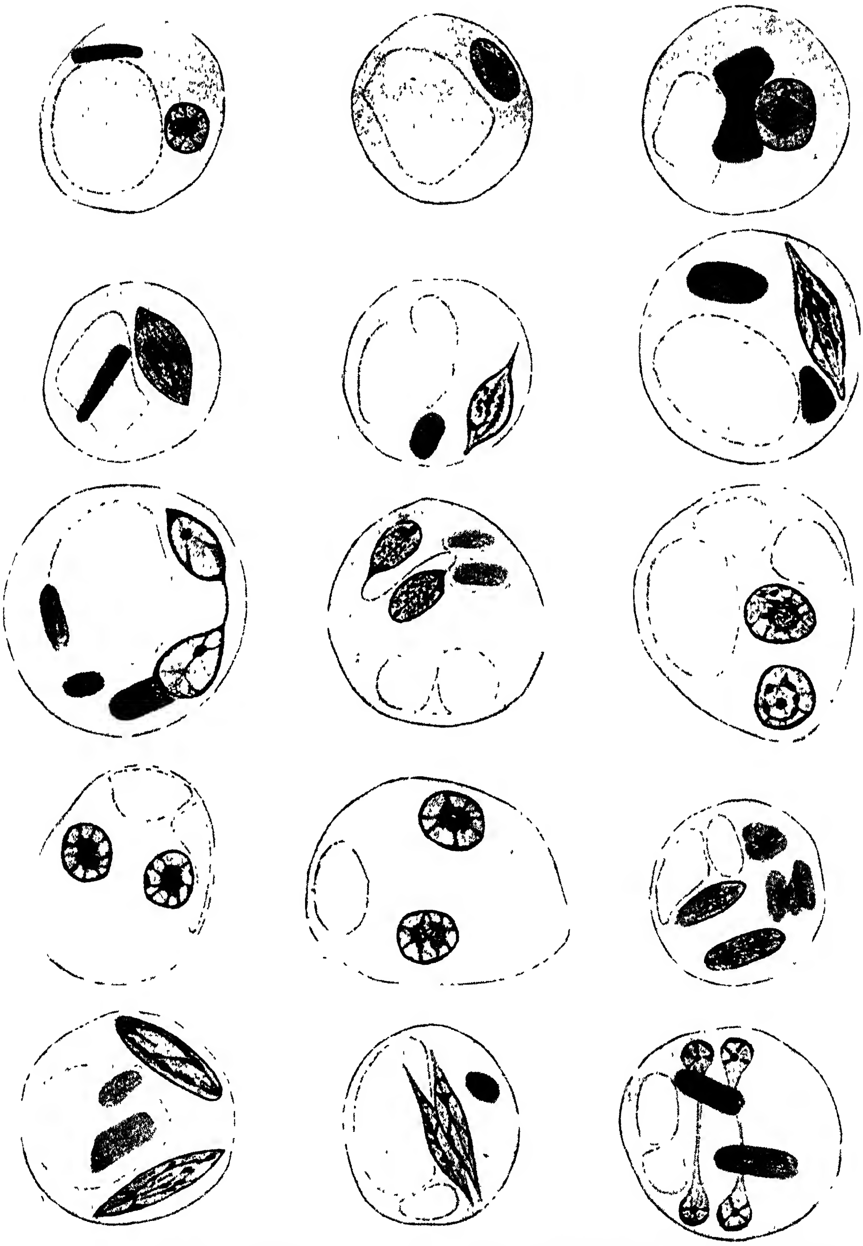


FIG. 57.—DIVISIONS OF THE NUCLEI IN THE CYSTS OF *Entamœba histolytica* (× 2,000). (ORIGINAL.)

The deeply stained chromatoid bodies are shaded, while the limits of the cytoplasm and vacuoles are shown in outline.

karyosomes disappear, and there cannot be detected any structures like centrosomes at the apices of the spindle. When the nuclear membrane commences to divide, karyosomes of the daughter nuclei reappear.

A modification of the preceding type of division is seen in an amœba described by Dobell (1914*a*) under the name *Amœba lacertæ*. The amœba is a common parasite of the intestine of *Lacerta muralis* and other lizards. In the resting condition the nucleus consists of a nuclear membrane and large central karyosome, in which all the chromatin of the nucleus is said to be aggregated (Fig. 58). When nuclear division commences, coarse granules of chromatin can be distinguished in the karyosome. These

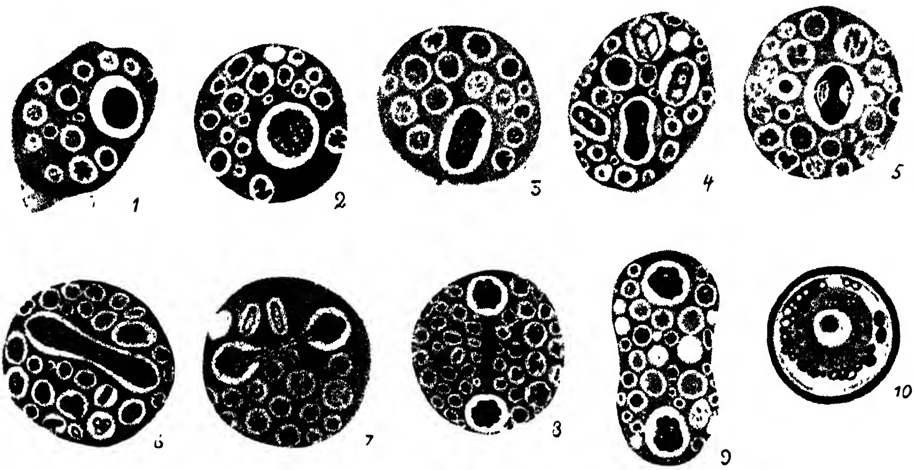


FIG. 58.—NUCLEAR DIVISION IN *Vahlkampfia dobelli* (*Amœba lacertæ*, DOBELL, 1914) (×2,000). (AFTER DOBELL, 1914.)

1. Ordinary form with nucleus containing large karyosome.
2. Karyosome breaking into granules.
3. Elongation of karyosome and arrangement of chromatin granules in meridional lines.
4-7. Elongation and constriction of karyosome.
8-9. Completion of nuclear division and commencing division of cytoplasm.
10. Encysted form.

become finer and arranged in meridional lines on the surface of the karyosome, which now becomes elongated, as does also the nuclear membrane. On the surface of the elongated karyosome granules of chromatin are arranged in longitudinal rows, and some indication of fibres can be detected. The granules gradually collect at the two poles of the karyosome, which itself becomes constricted at its centre and finally divided into two parts. This is followed by constriction and division of the nuclear membrane, which has persisted throughout the division process. The daughter karyosomes contract to the spherical form, while the granules of chromatin unite to form larger granules. During this division the

essential features are the appearance in the karyosome of granules which become irregularly arranged in longitudinal rows on the fibres which appear in the elongating karyosome. Spindle fibres are thus produced, but the chromatin granules do not unite to form chromosomes, nor is an equatorial plate developed. Nothing in the nature of a centrosome is present.

The stages in the division of *Amœba hyalina* (*Hartmannella hyalina*), described by Hartmann and Chagas (1910*b*), illustrate the type of division in which a centriole is supposed to be present (Fig. 59). The resting nucleus is described as having a centriole within the karyosome. When

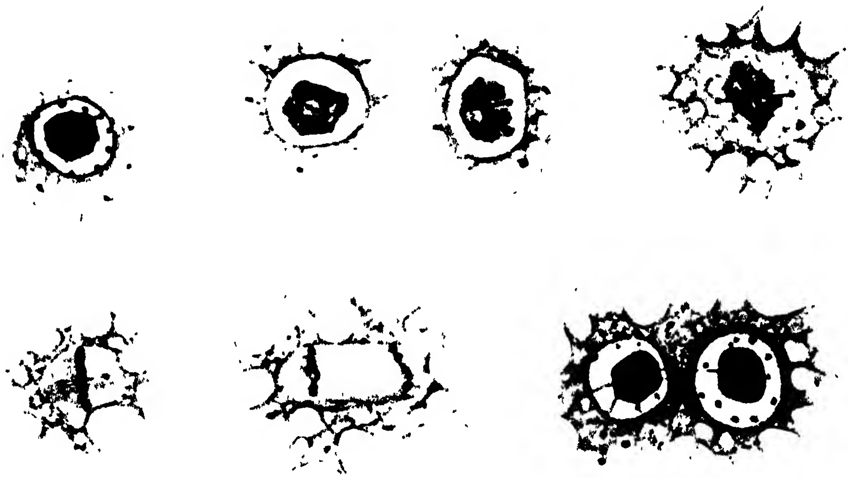


FIG. 59.—STAGES IN MITOTIC DIVISION OF NUCLEUS OF *Hartmannella hyalina*, IN WHICH IT IS SUPPOSED THAT A CENTRIOLE FUNCTIONING AS A CENTROSOME IS PRESENT (×3,700). (AFTER HARTMANN AND CHAGAS, 1910.)

division commences the centriole divides, and as the two halves separate they are connected by a centrodesmose. The chromatin of the karyosome breaks up into granules, which become arranged as chromosomes in an equatorial plate. Each daughter centriole has now taken up a position at the apex of the spindle-shaped nuclear membrane, within which is a system of exceedingly fine spindle fibres. The daughter plates of chromosomes are formed, and these move towards the poles of the spindle. Finally, the chromosomes at each end run together to form the karyosome, in which the centriole is included, while the intermediate part of the spindle disappears. It is by no means clear that the above is an accurate description. Other observers who have investigated the nuclear division of this or similar amœbæ have failed to detect the centrioles (Fig. 89).

Arndt (1924), in describing the nuclear division of *Hartmannella*

klitzkei, which, according to him, takes place by typical mitosis with extranuclear centrosomes, states that he has been able to demonstrate similar centrosomes in four species of *Hartmannella*. The centrosome, which does not originate from an intranuclear centriole, is easily overlooked, and requires very special technique for its demonstration (Fig. 60). In the resting nucleus it lies against the outer surface of the nuclear membrane, and when division commences it divides into two daughter centrosomes, which take up positions at the poles of the spindle. It is evident, in the light of these observations, that the granule described as a centriole

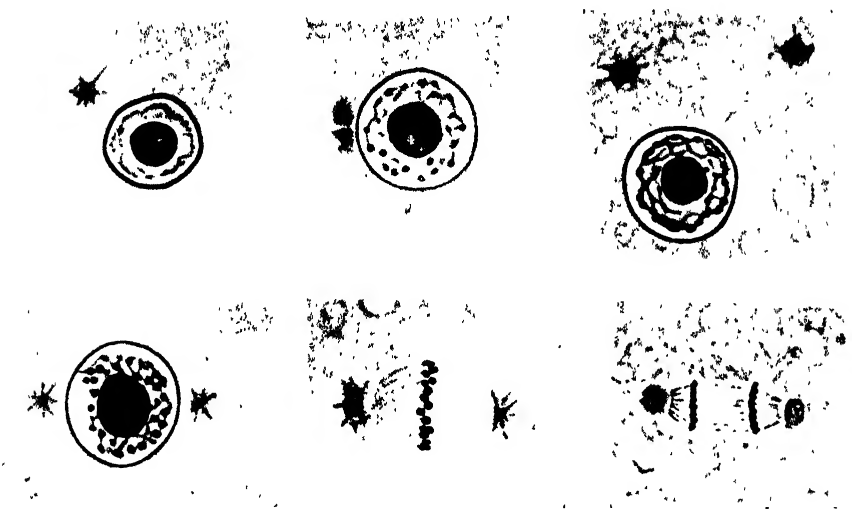


FIG. 60.—STAGES IN THE NUCLEAR DIVISION OF *Hartmannella klitzkei* TO SHOW THE PRESENCE OF THE EXTRANUCLEAR CENTROSOME AS REVEALED BY MANN'S STAIN (×2,500). (AFTER ARNDT, 1924.)

by Hartmann and Chagas cannot be a centrosome, and that the cases of mitosis which have been recorded as taking place without centrosomes require reinvestigation.

Another type of nuclear division which is distinct from those described above occurs in amœbæ belonging to the genera *Vahlkampfia* and *Dimastigamœba* (Fig. 61). The resting nucleus has a large central karyosome and peripheral chromatin in the form of fine granules within the nuclear membrane. The nuclear membrane persists throughout nuclear division, during which the karyosome becomes elongate and then dumb-bell-shaped, and finally constricted into two daughter karyosomes. These may remain connected by a fibre or centrodesmose, which in some cases can be seen to unite two granules which are embedded in the dense daughter karyosomes. Between the two karyosomes and surrounding the centro-

desmose are spindle fibres, at the equator of which chromosomes become arranged. These are formed from the peripheral chromatin granules of the nucleus, and possibly some which have separated from the karyosome. The chromosomes split to form two plates, which move towards the daughter karyosomes. The centrodesmose and the spindle fibres disappear, while the nuclear membrane is divided by constriction. Two daughter nuclei, each with a larger central karyosome and peripheral chromatin granules, are reconstructed.

In many cases, as, for instance, in trypanosomes and allied flagellates, in which the nucleus consists of a nuclear membrane containing a large central karyosome, all that can be detected in nuclear division is the elongation of the nuclear membrane, within which the karyosome becomes drawn out and finally dumb-bell-shaped. The narrow intermediate portion may be quite short, or it may be very much drawn out. In either case it finally disappears, leaving two daughter karyosomes. By constriction and division of the nuclear membrane two daughter nuclei are formed (Fig. 156). It is maintained by the advocates of the centriole theory that the narrow intermediate portion in the dumb-bell stage represents a centrodesmose connecting two daughter centrioles which are lodged in the daughter karyosomes. Certain appearances which are sometimes seen might lend support to this view. Occasionally, a dividing nucleus may be seen, in which a small granule is situated at each end of the elongated nuclear membrane. These are connected by a fine fibre, at the centre of which the still intact karyosome lies. Such an arrangement might be interpreted on the supposition that the centriole within the karyosome has divided prematurely, and that the two daughter centrioles have passed out of the karyosome, which has not yet shown any sign of division. At a later stage the karyosome divides, and the two daughter karyosomes pass to the ends of the nucleus and again enclose the centrioles. Such appearances, however, are unusual, and may be merely accidental arrangements of chromatin granules. Hartmann and Nöller (1918) have given another account of the nuclear division in *Trypanosoma theileri* (Fig. 156). They maintain that the apparently elongated karyosome is really a spindle, at each apex of which is a centriole, and that fine granules of peripheral chromatin form chromosomes which become arranged as an equatorial plate. Mitotic divisions of trypanosome nuclei have been described also by Chagas (1909) and Nieschulz (1922*b*).

As already intimated, the Protozoan nucleus sometimes contains a body which is entirely devoid of chromatin, and appears to consist of plastin material alone. In nuclear division it may disintegrate and disappear, to be re-formed again in the daughter nuclei. In some cases, however, it divides into two parts, which pass to the poles of the spindle with the

daughter chromosomes, and finally enter the daughter nuclei. Bodies of this kind have been described by Reichenow (1921) in *Karyolysus*, and the writer has seen them in *Hepatozoon balfouri* (Fig. 35). These plastin bodies are not essentially different from karyosomes, which consist mainly

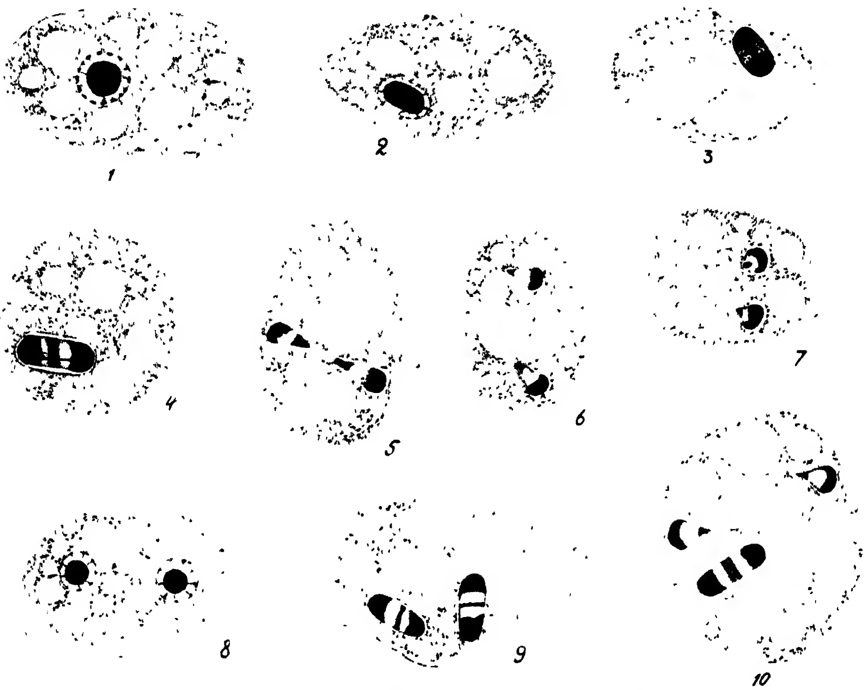


FIG. 61.—AMŒBOID PHASE OF *Dimastigamœba gruberi* FROM CULTURE ON AGAR PLATE SHOWING METHOD OF NUCLEAR DIVISION (× *ca.* 1,350). (ORIGINAL.)

1. Usual type of amœba.
2. Commencing nuclear division. The karyosome has become elongate and granular.
3. The karyosome has become dumb-bell-shaped and the nucleus is filled with granules.
4. There is an equatorial plate of dividing chromosomes, and the dividing karyosome has formed the pole caps, which are still united by a fibre (centrodesmose).
5. The daughter chromosomes have become aggregated, and are passing towards the pole caps, which have lost the connecting fibre. Each pole cap has a central granule.
6. The nucleus has divided and each half is retracting.
7. Slightly later stage with daughter nuclei still further retracted.
8. Form with two reconstituted nuclei.
9. Form with two nuclei in division: equatorial plate stage.
10. Form with two nuclei in different stages of division.

of achromatic material. In nuclear division the plastin substance, whether it be regarded as a karyosome or not, may divide into two parts, one of which goes to each daughter nucleus, as in *Dimastigamœba* (Fig. 61), or it may break up and disappear as a single body, to re-form in the daughter nuclei, as in *Hartmannella* (Fig. 56).

When reproduction by binary fission occurs, division of the nucleus is followed by division of the body of the organism into two parts. When multiplication by schizogony takes place, or when a number of gametes are produced, the nucleus divides into two, these into four, and so on, till the requisite number is reached. The multinucleated organism then buds from its surface a number of daughter individuals. The repeated divisions of the nuclei frequently take place by mitosis, especially when they are multiplying to form gamete nuclei, as in the case of gregarines or coccidia. It sometimes happens that before the spindle of one division

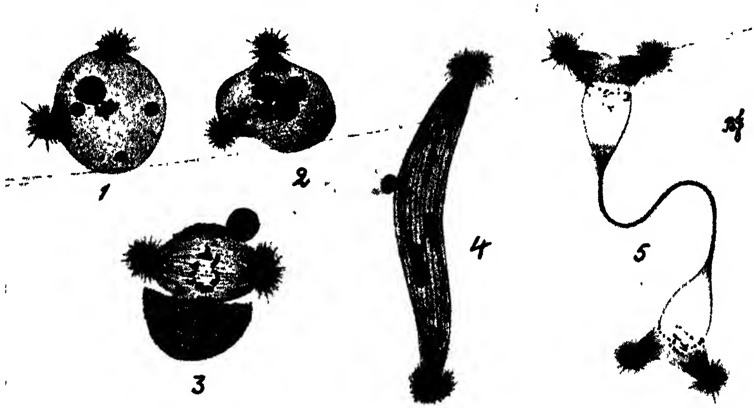


FIG. 62.—FIRST NUCLEAR DIVISION IN ONE OF A PAIR OF ASSOCIATED MONOCYSTID GREGARINES (*Monocystis* SP.) OF THE EARTH-WORM. (AFTER BRASIL, 1905.)

1, 2. Two centrosomes are present, the nucleolus is breaking up, while the chromatin has collected at the centre of the nucleus (× 900).
3. The spindle has formed, the nucleus has been extruded, and chromosomes are found at the equator of the spindle (× 900).
4. The chromosomes have divided and are passing to the poles of the spindle, where the centrosomes have already divided for the succeeding division (× 900).
5. Though the nuclei have not been definitely reconstituted, the spindles for the next division have formed (× 800).

has disappeared the two asters and the centrosomes, if these be present, divide again, so that two asters are formed at each end of the spindle. These may separate and form a new spindle between them, so that when the daughter chromosomes reach the pole of the original spindle they are already at the equator of a new one. In this manner very complicated poly-aster figures may arise. Precocious formation of daughter asters while the original spindle is still present has been shown to occur in a gregarine (*Monocystis*) of the earth-worm by Brasil (1905) (Fig. 62). Very complicated poly-aster figures similarly occur during nuclear

multiplication in species of *Aggregata*, as described by Moroff (1908) and other observers (Fig. 63).

The main types of nuclear divisions of Protozoa may be thus classified:

1. Mitotic division with centrosomes, asters, achromatic spindle, chromosomes, equatorial plates, and all the stages seen in the typical nuclear division of higher animals. The nuclear membrane may or may not persist during division. A nucleolus or plastin body, if present, may be divided into two parts, one of which goes to each daughter nucleus, or it may break up and disappear, the daughter nuclei re-forming their nucleoli when division is approaching completion.

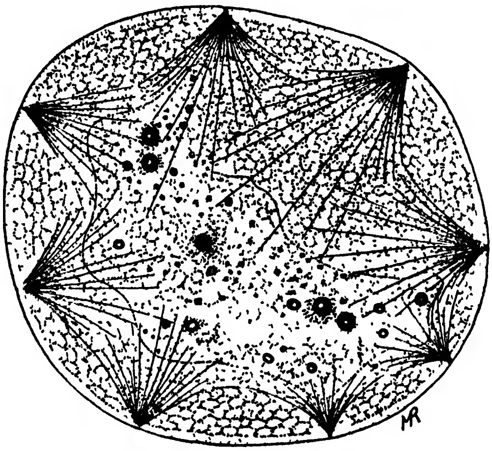


FIG. 63.—POLY-ASTER FIGURE RESULTING FROM SUCCESSIVE NUCLEAR DIVISIONS IN MALE GAMETOCYTE OF *Aggregata jacquemeti* (× 750). (FROM MINCHIN, 1912, AFTER MOROFF.)

2. Mitotic division of the above type, except that centrosomes and asters have not been detected.

3. Division in which there is formed within the nuclear membrane a spindle upon which chromatin granules are irregularly arranged. There are no asters or centrosomes. It is possible that the granules of chromatin, though not arranged as an equatorial plate, are actually chromosomes, which divide into daughter chromosomes as they do in the preceding types of division. The karyosome may divide into daughter karyosomes, or break up to be re-formed in the daughter nuclei.

4. Division in which the large central karyosome elongates and becomes constricted. The two halves move to the ends of the elongating nuclear membrane to form the pole caps between which a spindle is formed. The peripheral chromatin becomes arranged as chromosomes in an

equatorial plate. The pole caps become the karyosomes of the daughter nuclei

5. Division in which the karyosome becomes elongated and divided within the nuclear membrane without development of spindle fibres or chromosomes. This type of division is seen in the nuclei of small organisms, and it is probable that it is actually similar to type 4, the spindle fibres and chromosomes escaping detection owing to their minuteness.

In those cases in which a centrosome is not present, some observers claim that its place is taken by an intranuclear centriole.

BEHAVIOUR OF CHROMOSOMES DURING SYNGAMY.

Reference has already been made to the nuclear changes which occur during the development of the ovum and the spermatozoon, and it has been pointed out that the chromosome number of the zygote nucleus is not doubled as a result of syngamy owing to the fact that after meiosis the nuclei of the uniting gametes contain half the normal number of chromosomes. Several instances of similar reduction divisions of Protozoan nuclei, whereby the number of chromosomes is halved, have been recorded.

Muslow (1911) gives a clear account of a supposed reduction division in *Monocystis rostrata* (Fig. 64). The nuclei of the two gregarines which enter the gametocyst multiply by repeated mitotic divisions in which eight chromosomes are present, as noted on p. 92. Eventually, after nuclear division has ceased, a number of gametes are budded off from each gregarine, and these unite in pairs and their nuclei fuse. During the last nuclear division, whereby the gamete nuclei are formed, though eight chromosomes appear on the equatorial plate, when the daughter plates are formed, there is no splitting of the chromosomes, as has occurred in previous divisions, but the eight chromosomes are separated into two groups of four, which move towards the poles of the spindle. It was noted also that the eight chromosomes composing the equatorial plate consisted of four pairs of homologous chromosomes, the members of each pair differing from those of other pairs, and that one of each pair entered each daughter plate of chromosomes. This last division, which gives rise to the nuclei of the gametes, is thus a true reduction division or meiosis, like that which occurs in the production of gametes in higher animals. When the gametes unite, the nucleus of the zygote, receiving four chromosomes from each gamete nucleus, again has eight chromosomes or four pairs of homologous chromosomes.

In the case of *Diplocystis schneideri*, a gregarine of the cockroach,

Dobell and Jameson (1915) have given a description of a reduction division which differs from that of Muslow. According to these observers, during all the division stages of the nuclei, including the last division which gives rise to the gamete nuclei, there are three chromosomes which divide to form the chromosomes of the daughter nuclei (Fig. 65). The nuclei of the gametes thus have three chromosomes, as do the nuclei of the preceding stages. When the gametes unite and their nuclei fuse, the zygote nucleus has six, or double the number of chromosomes found at other stages. The zygote nucleus now proceeds to division, and it is in this division that the reduction occurs, three of the six chromosomes passing to each daughter nucleus. At all subsequent division stages of the nuclei the three chromosomes are divided. In Muslow's account of *Monocystis rostrata* it was

1 2 3 4

FIG. 64.—LAST NUCLEAR DIVISION IN ONE OF TWO ASSOCIATED GREGARINES, *Monocystis rostrata*, TO SHOW THE REDUCTION OF THE CHROMOSOME NUMBER FROM EIGHT TO FOUR IN THE GAMETE NUCLEI (× 5,000). (AFTER MUSLOW, 1911.)

1. Eight chromosomes in nucleus. 2. Eight chromosomes arranging themselves in pairs.
3. Separation of the individual chromosomes of each pair.
4. Four chromosomes moving to each pole of the spindle to form the gamete nuclei.

during the last nuclear division in the production of gamete nuclei that the number of chromosomes was halved, whereas in Dobell and Jameson's account of *Diplocystis schneideri* the reduction does not occur at this stage, but at the first division after the zygote nucleus has been formed. According to Muslow, the haploid number of chromosomes of *Monocystis rostrata* is four, and occurs in the gametes, while all other nuclei have the diploid number of eight chromosomes; on the other hand Dobell and Jameson in *Diplocystis schneideri* find that the diploid number six occurs only in the zygote, all other stages showing the haploid number three. The latter observers have noted the same condition in the case of the coccidium *Aggregata eberthi* (Fig. 66). In this parasite, during schizogony nuclear divisions occur in which six chromosomes appear in the equatorial plate, and they all divide so that the daughter nuclei have

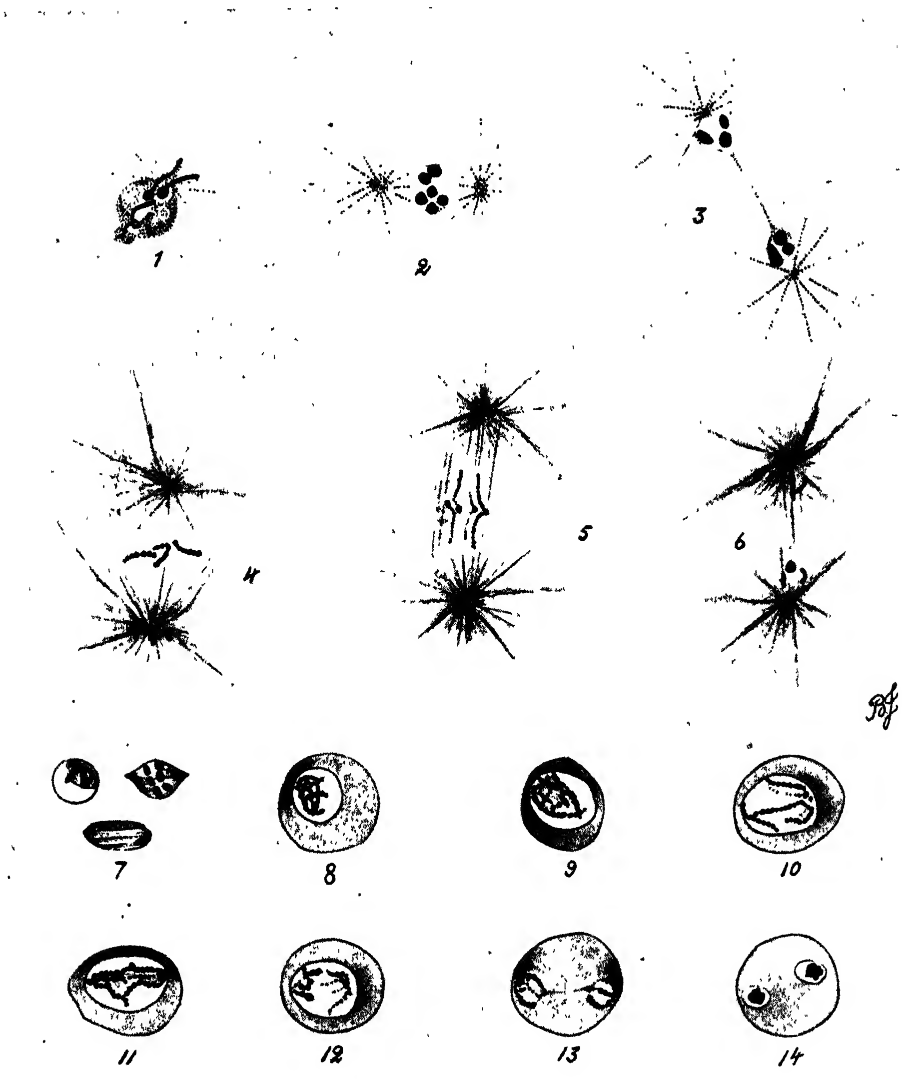


FIG. 65.—NUCLEAR DIVISION IN THE GREGARINE, *Diplocystis schneideri*, TO ILLUSTRATE THE REDUCTION IN THE CHROMOSOMES IN THE FIRST NUCLEAR DIVISION IN THE ZYGOTE (× 2,500). (1-3 AFTER DOBELL AND JAMESON, 1916; 4-14 AFTER JAMESON, 1920.)

1-3. First division in the gregarine, showing division and separation of the three chromosomes.
4-6. Third division in the gregarine, in which three chromosomes again divide.
7. Last nuclear division to form the gamete nuclei: three chromosomes again divide; there is no reduction. 8-9. Zygote nucleus with six chromosomes.
10. The six chromosomes arranged in three pairs.
11, 12. Separation of the chromosomes in two groups of three (reduction).
13, 14. Reconstitution of two nuclei, each with three chromosomes.

each six chromosomes. Finally, male and female gametocytes which give rise to male and female gametes are formed. The nucleus of the male or microgametocyte multiplies by repeated divisions in which the series of six chromosomes are present (Fig. 66, A to D). They are filamentous except when arranged as the equatorial plate, when they are contracted and more or less spherical, though maintaining the same relations as regards size. At the equator of the spindle the chromosomes divide by constriction, and the two groups of six daughter chromosomes separate and become filamentous again. By repeated divisions of this kind, in which the daughter asters divide before actual nuclei are formed, very complicated poly-aster figures are produced. Eventually, as in the schizont, nuclei which lie on the surface are constituted, and from them the microgametes are formed. The latter are elongate bodies provided with two flagella at the anterior end (Fig. 376). Meanwhile, certain merozoites of the female line have become female- or macro-gametocytes. A complicated series of changes takes place in the nucleus. The nucleolus or karyosome is thrown out, the nuclear membrane disappears, and a series of six long chromosomes appears (Fig. 66, E). Finally, a fertilization spindle is formed, on which the chromatin of the female nucleus is arranged in the form of granules (see p. 873). The chromatin of the male nucleus, derived from the microgamete, now enters the spindle, which retracts to form the zygote nucleus (synkarion). This nucleus now proceeds to division by mitosis, and the chromosomes are reconstituted (Fig. 66, F to K). It is found that there are twelve of these—a series of six pairs, the two constituting each pair being equal in size. Undoubtedly one chromosome of each pair is derived from the microgamete nucleus and one from the macrogamete nucleus. The twelve chromosomes now pass to the equator of the spindle and become globular in form, and the two constituents of each pair now unite, giving a stage in which there are only six double chromosomes (Fig. 66, G). The union, however, is not permanent, for separation takes place, and one chromosome of each pair passes to one pole of the spindle, while the other goes to the opposite pole (Fig. 66, H). In this process there has been no division of the chromosomes, so that in each daughter group there are only six chromosomes, whereas in the zygote nucleus (synkarion) there were twelve. The first division of the synkarion is thus a true reduction division, whereby the original number of six is regained. It will thus be seen that in every stage of development of this parasite the nuclei have six chromosomes, except in the synkarion formed by union of the male and female nuclei, in which there are twelve. The daughter groups of six chromosomes resulting from the division of the synkarion now proceed to division again, but, as in the case of the nuclear multiplication in the schizont and microgametocyte, at each

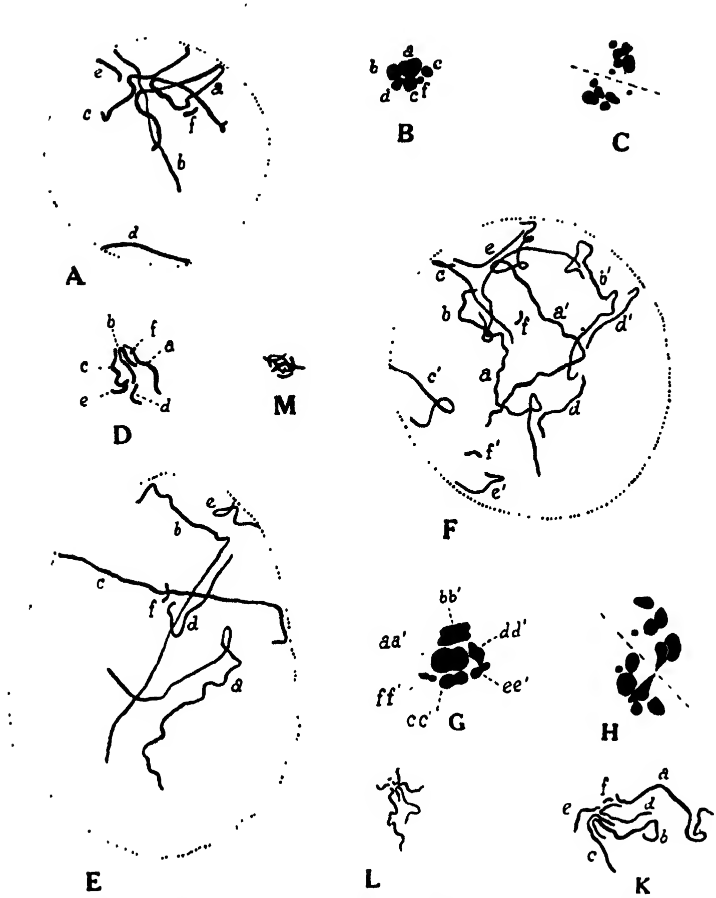


FIG. 66.—CHROMOSOMES OF *Aggregata eberthi* (× 2,000). (AFTER DOBELL AND JAMESON, 1915.)

A. Nucleus of male showing six long chromosomes at prophase stage of first division.
B. Later stage of first division of nucleus of male: the chromosomes have become compact and are arranged as an equatorial plate.
C. Later stage each chromosome has divided to give rise to two groups of six daughter chromosomes.
D. One of the groups of six daughter chromosomes arising from first division of male nucleus elongating to form the chromosomes of one of the daughter nuclei.
E. Nucleus of female before fertilization, showing six long chromosomes.
F. Chromosomes in zygote nucleus: early stage of first division, showing twelve chromosomes, six (*a*-*f*) derived from the male, and six (*a*′-*f*′) from the female.
G. Chromosomes in zygote nucleus· equatorial plate stage of first division; the twelve chromosomes have contracted and become associated as six double chromosomes.
H. Chromosomes in dividing zygote nucleus the individual chromosomes of each pair have separated, giving rise to two groups of six.
K. End of first division of the zygote nucleus; one of the groups of six chromosomes, which have elongated, entering the daughter nucleus.
L. Group of six daughter chromosomes on spindle of a later nuclear division of the zygote.
M. Group of six chromosomes forming equatorial plate at second division of the spore nucleus.

division the six chromosomes divide, so that each daughter nucleus has six chromosomes (Fig. 66, L and M). Eventually, a large number of nuclei are formed. These arrange themselves on the surface of the cytoplasm, which segments into a number of sporoblasts.

An exactly comparable process has been described by Reichenow (1921) in the case of hæmogregarines of the genus *Karyolysus* (Fig. 457). Here the haploid number of chromosomes is four, and these occur in nuclei of all stages except those of the zygotes, which have the diploid number of eight. When the zygote nucleus divides, four closely united pairs of chromosomes occur at the equator of the spindle. One chromosome of each pair then passes towards the pole of the spindle, so that the resulting daughter nuclei have again only four (see p. 1098). These accounts agree in that the reduction division occurs at the division of the zygote nucleus, and not, as Muslow maintains, in the last division which gives rise to the gamete nuclei. It seems highly improbable that *Monocystis rostrata* would differ from other gregarines or coccidia in this respect, and Dobell and Jameson have suggested that possibly Muslow was dealing with a mixed infection of two gregarines, one of which has a chromosome number of four and the other of eight, and that what he considered to be the reduction division of the form with eight chromosomes was in reality the ordinary division of the form with four chromosomes.

The nuclear division during the vegetative reproduction by binary fission, the formation of gametes, and their maturation in the Heliozoon *Actinophrys sol* has been the subject of detailed study by Bělař (1923), as mentioned above. The organism reproduces by simple division. Finally, encystment occurs and the uninucleated individual within the cyst divides to form two gametes (Fig. 50). The nucleus of each gamete divides and one of these degenerates. The remaining nucleus then divides, and one of the resulting nuclei degenerates. There have thus been two maturation divisions of the gamete nuclei. Conjugation of gametes then occurs. During vegetative reproduction the nucleus divides without centrosomes by mitosis, while retaining its nuclear membrane. When the chromosomes, which number forty-four, first appear during nuclear division they are thread-like, but as the equatorial plate stage is reached they become much shortened, and finally roughly spherical, in which condition they divide to form daughter chromosomes. When the encysted individual divides to form the two gametes, the nuclear division is of the same type as that occurring during the ordinary vegetative reproduction. The forty-four long chromosomes become arranged in twenty-two pairs, the members of each pair being closely applied to one another. Finally, when the equatorial plate stage is reached, there are present at the equator of the spindle twenty-two pairs of more or

less rounded chromosomes. Each chromosome splits into two, so that the daughter plates and finally the daughter nuclei also contain twenty-two pairs of chromosomes. Each resulting nucleus then undergoes two maturation divisions. In the first of these at the equatorial plate stage there are twenty-two pairs of rounded chromosomes, but when the daughter plates form the chromosomes do not split, as in the preceding nuclear division. One chromosome of each pair passes to each daughter plate, which thus contains only twenty-two chromosomes instead of twenty-two pairs. The process is similar to that shown at Fig. 4, except that in the place of the four chromosomes there are forty-four. Of the resulting nuclei, one degenerates and the survivor divides by mitosis as before. During this division twenty-two chromosomes appear at the equator of the spindle, and each divides, so that each resulting nucleus has twenty-two chromosomes. After union of the gametes, the zygote nucleus has forty-four chromosomes. During all these divisions the chromosomes are long filaments at the commencement of nuclear division, but they gradually retract and finally become roughly spherical, in which form they are arranged as the equatorial plate.

In connection with the conjugation of ciliates, similar reduction processes have been described. In these Protozoa, as explained above, it is only the micronucleus which takes part in syngamy, the macronucleus degenerating. The micronucleus in one individual divides to form two nuclei, and these again to form four. Of these four, three degenerate. The remaining one divides again, so that each of the two associated ciliates contains two nuclei. One of the nuclei in each individual now passes over to the other and unites with the stationary nucleus, after which the ciliates separate. Here, again, if the number of chromosomes in the uniting nuclei has not been reduced, it is evident the zygote nuclei will have double this number. Several observers have maintained that the first of the three divisions of the micronucleus is really a reducing division. Hertwig (1889) noted that in *Parameccium aurelia*, the nucleus of which has a large number of chromosomes during division, the nuclei which unite have approximately half the number of chromosomes seen in the ordinary divisions of the micronucleus during reproduction by fission. Calkins and Cull (1907), in the case of *Paramecium caudatum*, noted that the number of chromosomes in the ordinary dividing nucleus is about 165. During the first two divisions of the micronucleus during conjugation there is a reduction in the number to about half this. On account of their large number it is difficult to count the chromosomes accurately. Prandtl (1906) found that in *Didinium nasutum* the first division of the micronuclei during conjugation was associated with the reduction of the chromosomes from sixteen to eight. In *Collinia*

branchiarum, Collin (1909) described a reduction of from six to three (Fig. 495, 5 and 6), while Enriques (1908*a*) in *Chilodon uncinatus* saw a reduction of four to two, and (1907) in *Opercularia coarcta* a reduction of sixteen to eight (p. 1174). In all these cases the conjugating or gamete nuclei possess half or the haploid number of chromosomes, while the nuclei resulting from the union of the gamete nuclei have the full or diploid number, which is maintained at all subsequent divisions. This is the reverse of what occurs in the gregarines and coccidia, as described by Dobell and Jameson, and Reichenow.

In connection with the process of union of gametes many so-called reduction or maturation processes have been described. In *Eimeria schubergi*, Schaudinn (1900), for instance, described as a maturation process the breaking up and extrusion from the nucleus of the macrogamete of the large karyosome (Fig. 337, 11). From what has been said above of the reduction division of the nuclei of coccidia, gregarines, and ciliates, it seems highly improbable that such a process is a reduction at all. In the case of *Cyclospora caryolytica*, another coccidium, Schaudinn (1902) described the macrogamete nucleus as dividing twice, one of the products of each division degenerating (Fig. 341). This again is explained as a maturation process for the macrogamete nucleus before it is fertilized by the microgamete. A similar process is said to take place in the case of the parasites of malaria. The macrogamete, before fertilization in the mosquito's stomach, is supposed to extrude one or two polar bodies which contain some of the chromatin of the nucleus (Fig. 391, 16). In the case of the conjugation of the flagellate *Copromonas subtilis* described by Dobell (1908*b*), where two individuals fuse, before the union of the nuclei each nucleus is said to divide twice to form two reduction bodies which degenerate (Fig. 48). After this, the nuclei of the conjugating individuals unite. From what has been discovered during the past few years regarding the methods of reduction of the number of chromosomes in connection with the union of gametes in the Protozoa, it is evident that many of the processes previously interpreted as reduction or maturation divisions of the nuclei need to be re-examined in the light of what is now known. Till this has been done it is useless to speculate as to their meaning.

BLEPHAROPLASTS, PARABASALS, AND KINETOPLASTS.

It has been explained above that amongst the Mastigophora the axis of the flagellum is a filament (axoneme) which arises from a granule called the blepharoplast. When there are two or more flagella, there are a corresponding number of axonemes and blepharoplasts. The several

blepharoplasts, when more than one is present, are often so closely packed together that it may be difficult to distinguish them individually.

The blepharoplast may be situated upon the nuclear membrane, as in *Cercomonas*, or quite separate from it, as in the majority of other flagellates. It has already been shown above that certain observations tend to indicate that the blepharoplast is of nuclear origin. In certain stages a flagellate may lose its flagellum or flagella and become a rounded body with a single nucleus. When the flagellum is about to be re-formed, it is claimed that a granule separates from the karyosome of the nucleus and passes out into the cytoplasm through the nuclear membrane (Fig. 31).

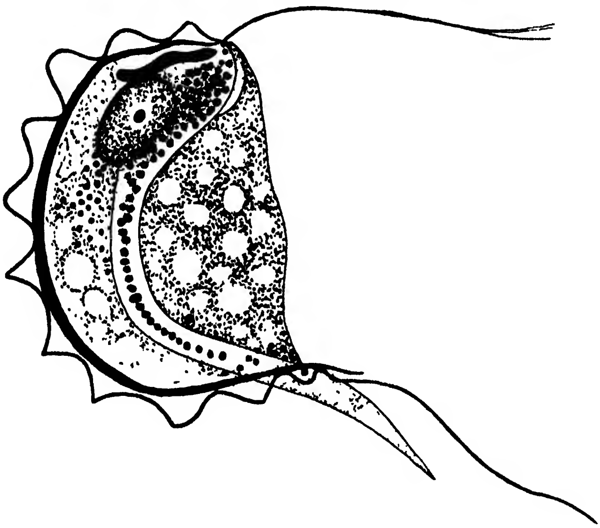


FIG. 67.—*Trichomonas augusta*, SHOWING THE SPIRAL PARABASAL BODY IMMEDIATELY ANTERIOR TO THE NUCLEUS ($\times$ *ca.* 2,500). (AFTER ALEXEIEFF, 1924.)

An axoneme is then formed from it as an outgrowth, and when the surface of the body is reached it takes with it a sheath of cytoplasm and becomes a flagellum.

In association with the blepharoplast, whether it is on the nuclear membrane or separate from it, there may occur one or more masses of a substance which stains deeply with many chromatin stains. To such bodies Janicki (1911) has given the name *parabasal* (see p. 53). The name *kinetoplast* is employed here to designate the compound structure consisting of a united parabasal and blepharoplast. Kinetoplasts are typically seen in trypanosomes and allied flagellates. Parabasal bodies have been described as occurring in *Trichomonas* by Janicki (1915), Wenrich (1921), and Alexeieff (1924), but they are only detected after special fixation—*e.g.*, osmic acid (Figs. 67 and 275).

When a flagellate is about to divide, the blepharoplast is usually the first structure to show any indication of division. It becomes elongated and constricted into two parts. Very often the two daughter blepharoplasts (or two groups of daughter blepharoplasts when several are present) remain connected by a fibre which may be called the *paradesmose*, as suggested by Kofoid and Swezy (1915), to distinguish it from the centrodesmose which unites the daughter karyosomes, or centrioles which are supposed by some observers to occur within the karyosome, during division (Fig. 272). As the blepharoplast elongates and divides and the daughter blepharoplasts separate, the parabasal also becomes elongated and divides. If several parabasals are present, without dividing individually, they separate into two approximately equal groups. The blepharoplast thus leads the way in division of the parabasal. It sometimes happens that the blepharoplast divides before the parabasal shows any signs of division. A figure may be produced in which the two daughter blepharoplasts are connected by a paradesmose, at the centre of which the still undivided parabasal lies. The parabasal now divides, and the two halves move towards the daughter blepharoplasts. There is some resemblance to mitosis in this type of division, which has been employed as an argument in support of the view that the blepharoplasts are centrosomes and that the kinetoplast is actually a nucleus. The parabasal, however, does not form chromosomes, nor are spindle fibres developed between the blepharoplasts, though some claim to have observed these structures during the division of the kinetoplast of trypanosomes. After the blepharoplast and parabasal have commenced to divide, the nucleus itself begins to show signs of division.

In flagellates like *Heteromita uncinata* and *Cercomonas longicauda*, in which the blepharoplast is on the nuclear membrane, a condition is seen in which the blepharoplast appears to function as a centrosome (Fig. 68). The blepharoplast upon the membrane divides, and the two halves separate. They finally take up positions at opposite poles of the nucleus, and a definite spindle is formed between them. The karyosome breaks up, and chromosomes appear at the equator of the spindle. The chromosome plate divides into two daughter plates which move towards the blepharoplasts. Finally, the nuclear membrane is divided, the chromosomes disappear, and with the formation of the karyosomes the nuclei are reconstructed.

It seems difficult to resist the conviction that in such a division the blepharoplast has fulfilled the function of a centrosome. Its behaviour, however, may be merely due to its position on the nuclear membrane, for in flagellates like *Parapolytoma satura*, described by Jameson (1914), in which the blepharoplast is separated from the nuclear membrane,

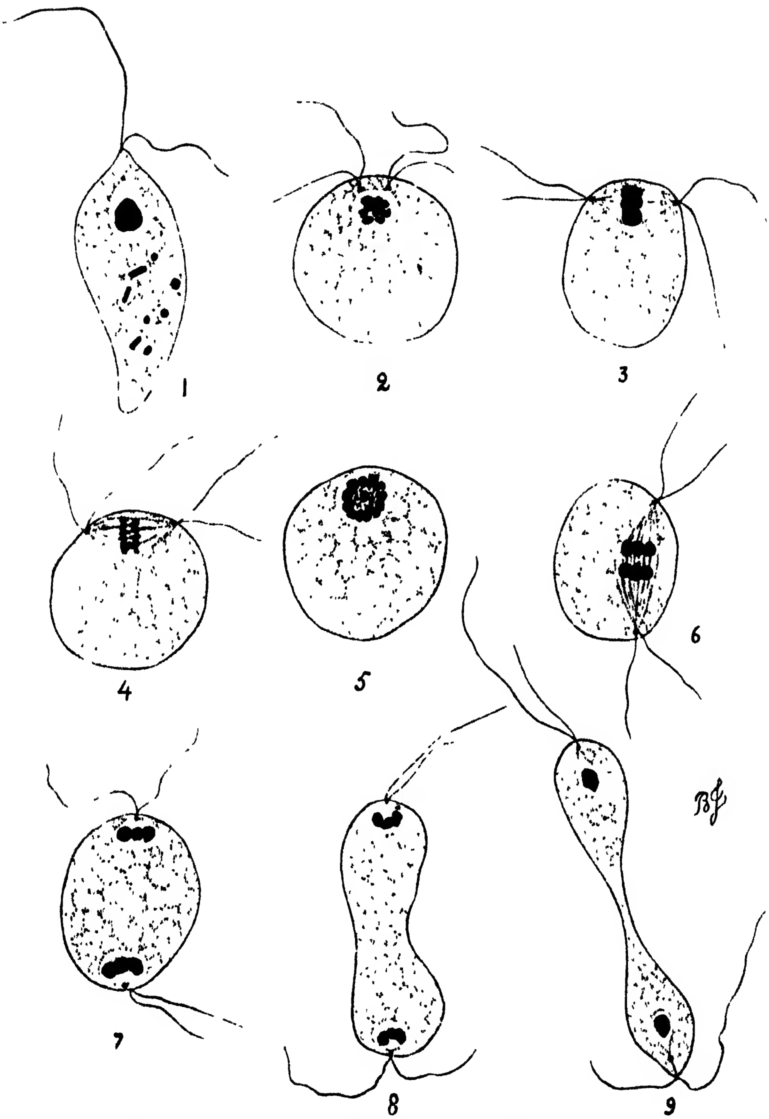


FIG. 68.—BINARY FISSION IN *Heteromita uncinata* (× 4,000). (ORIGINAL.)

1. Normal flagellate with blepharoplast on surface of nuclear membrane.
2. The flagellate has become rounded and its blepharoplast divided, while two new flagella have formed. The karyosome has broken up into granules.
3. The blepharoplasts occupy the poles of a spindle which has an equatorial plate of chromosomes.
4. The chromosomes have divided to form two daughter plates.
5. End view of the equatorial plate.
6. The daughter plates are separating.
7. Formation of two nuclei and the reconstruction of karyosome.
8. Commencing division of the flagellate.
9. The karyosome has re-formed and the flagellate is about to divide.

mitotic division of the nucleus occurs without any centrosomes at the poles of the spindle. Instances are known, however, in which the blepharoplasts which are separate from the nucleus occupy during nuclear division positions upon the spindle which centrosomes would be expected to occupy. Such an example is seen in the division of *Oikomonas termo* described by Martin (1912) (Fig. 135).

In the case of *Prowazekella lacertæ*, which has an axoneme originating in a blepharoplast on the nuclear membrane, the nucleus has one or more parabasals surrounding it. When division of the nucleus takes place, the daughter blepharoplasts occupy the poles of the spindle and mitotic division takes place, as in *Heteromita* and *Cercomonas*. The parabasal, if there is a single one outside the nucleus, becomes elongated and divided into two parts, one of which passes to each daughter nucleus. When there are several parabasals they separate into two groups without dividing individually, very much like the behaviour of mitochondria during division of spermocytes in the process of spermatogenesis (Fig. 254, *s*-*x*).

The function of the centriole in nuclear division has been discussed above. It will be seen that in *Heteromita uncinata*, *Cercomonas longicauda*, and other forms in which the blepharoplast occurs on the nuclear membrane, and in certain cases where it is separated from the membrane, the daughter blepharoplasts occupy during nuclear division the same positions that the daughter centrioles are said to occupy. It is claimed that as the centriole is functionally a centrosome, the blepharoplasts of flagellates must also be centrosomes. It is further assumed that, in those cases in which the blepharoplast occupies a position in the cytoplasm apart from the nucleus, it represents a centriole or centrosome which has left the nucleus or is the result of division of the centrosome into two parts, one of which remains in the nucleus and still functions as a centrosome during its division, while the other has left the nucleus to become a blepharoplast.

The whole subject of the relation of blepharoplasts to centrosomes is a very complex one, and depends largely on the exact definition of a centrosome. Some observers definitely assert that the blepharoplast is a centrosome. Minchin (1914), for instance, stated that in his opinion it was a well-established fact that in a great many cases blepharoplast and centrosome were one and the same body. It seems difficult to doubt this in view of the fact that in the developing spermatozoon of higher animals the axial filament of the tail which corresponds with an axoneme is known to be formed as an outgrowth from the centrosome. In fact, the tail with its axial filament arising from the centrosome is exactly comparable with the flagellum with its axoneme and blepharoplast.

The question of the nature of the numerous blepharoplasts possessed

by the Hypermastigida and the basal granules of the cilia of Ciliophora, which are to all intents and purposes blepharoplasts, is still more difficult to answer.

Another point in connection with the blepharoplasts of flagellates must be mentioned. Many observers have described fibres which connect the blepharoplasts with the karyosome of the nucleus, and they suppose that these fibres represent centrodesmoses which were formed when the supposed intranuclear centriole divided off the blepharoplasts. As already remarked, when several blepharoplasts are present, they are usually packed so closely together that they cannot be distinguished individually. It not infrequently happens, however, that in certain individuals of any species of flagellate the blepharoplasts are more dispersed, so that it is possible to recognize the actual number present. Kofoid and Swezy (1920) have described a very complicated system of fibrillar connections between the various blepharoplasts of *Chilomastix*, and they introduce into their scheme a definite centrosome which they state is present upon the nuclear membrane and is connected by a fibre with one of the blepharoplasts (Fig. 69). If such a centrosome and system of fibres is present in this flagellate, it has at any rate escaped detection by most observers. The complicated system of fibres which they describe as being present, together with the karyosome, centrosomes, blepharoplasts, flagella, and other motor organs and marginal filaments of the cytostomal groove, they name the *neuromotor system*. This term has been extended by them to include the fibrillar structures which occur in other flagellates, such as the complex organisms parasitic in termites, while Sharp (1914) employs it for the fibrillar apparatus of the ciliate *Diplodinium ecaudatum* (Fig. 520). It is quite possible that some of the fibres have a motor function, but others appear to be merely supporting rods, while there is at present no direct evidence to prove that they are comparable to nerve fibrils which the name neuromotor suggests. In using the term "neuromotor system," groups of structures which are not necessarily homologous in different organisms have been united under one name. Kofoid and Swezy, for instance, homologized one of the fibres which support the margin of the cytostome of *Chilomastix*, the basal fibre of the undulating membrane in *Trichomonas*, and the two structures of unknown function which commonly occur in the posterior region of *Giardia* as parabasals. There seems to be no real evidence that these are in any way homologous with the true parabasals of other flagellates, and it is worthy of note that several observers have described what are probably true parabasals in certain species of *Trichomonas*.

The growth and the formation of new flagella are intimately bound up with the activities of the blepharoplast. When the blepharoplast of

a flagellate divides, the axoneme which arose from it remains, as a rule, attached to one daughter blepharoplast, while a new axoneme grows out

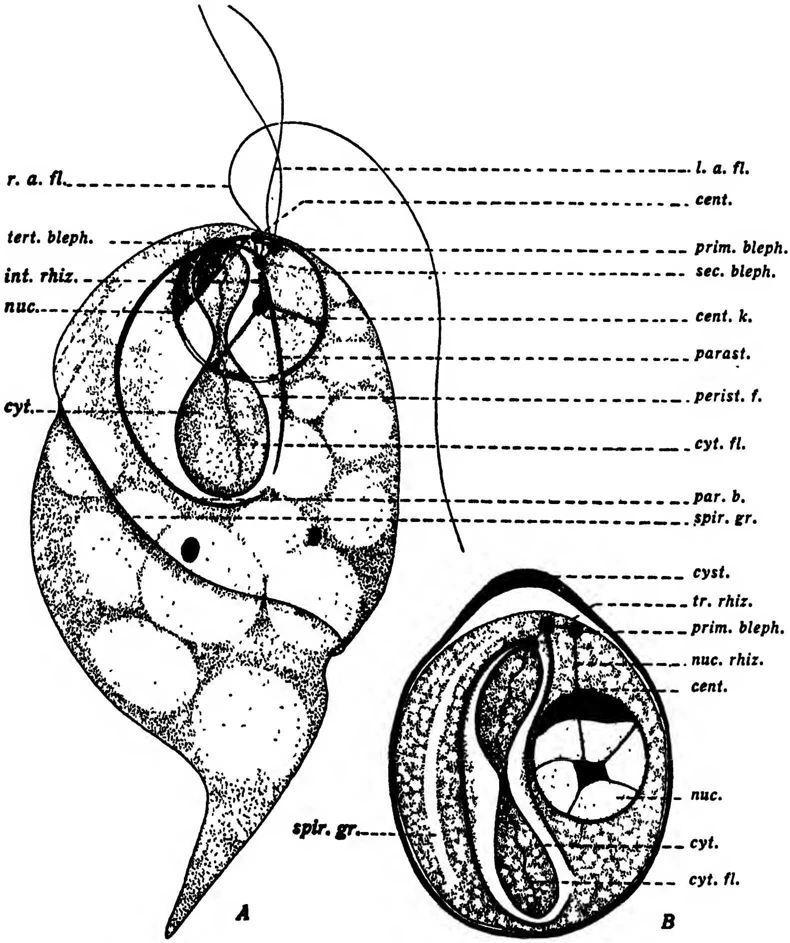


FIG. 69.—*Chilomastix mesnili:* FREE AND ENCYSTED FORMS, TO ILLUSTRATE THE STRUCTURES DESCRIBED BY KOFOID AND SWEZY (×6,370). (AFTER KOFOID AND SWEZY, 1920.)

A. Normal flagellate viewed from the ventral or oral side, and showing all the structures of the body. B. Cyst viewed from the ventral or oral side.

Cent., Centrosome; *cent.k.*, central karyosome; *cyst*, cyst wall; *cyt.*, cytostome; *cyt fl.*, cytostomal flagellum or undulating membrane; *int.rhiz.*, intranuclear rhizoplast; *l.a.fl.*, left anterior flagella; *nuc.*, nucleus; *nuc.rhiz.*, nuclear rhizoplast; *par.b.*, parabasal body; *parast.*, parastyle; *perist.f.*, peristomal fibre; *prim.bleph.*, primary blepharoplast; *r.a.fl.*, right anterior flagellum; *sec.bleph.*, secondary blepharoplast; *spir.gr.*, spiral groove; *tert.bleph.*, tertiary blepharoplast; *tr.rhiz.*, transverse rhizoplast.

from the other to form a new flagellum. When a group of blepharoplasts, in flagellates with more than one flagellum, divides into two groups, some

of the axonemes and flagella remain with one group and some with the other. There seems to be no regularity in their distribution. Those blepharoplasts which have no axonemes then form new ones. Very frequently, before the blepharoplast has actually divided, a new axoneme grows out from the part of the elongating blepharoplast which will become one of the daughter blepharoplasts. It may happen that the new axoneme actually passes into the cytoplasmic sheath of the old flagellum, so that finally longitudinal splitting of the flagellum occurs. In such a case division of the sheath of the flagellum alone takes place. It seems highly probable that in no case does an axoneme itself divide longitudinally. A new axoneme is invariably formed as a result of the outgrowth from the daughter blepharoplast. In cultures of *Leishmania* the flagellum is formed by outgrowth of the axoneme, which can usually be detected in properly stained specimens of the parasites as they occur in tissues (Fig. 192).

Certain structures other than axonemes take origin in granules, which are usually regarded as blepharoplasts. Thus, the two fibres which border the cytostomal groove in *Chilomastix* arise each from a granule or blepharoplast (Fig. 69). Similarly, the basal fibre of the undulating membrane in *Trichomonas* originates in a blepharoplast, and when division occurs a second basal fibre grows out from one of the daughter blepharoplasts into which the original one has divided. The axostyle of *Trichomonas* likewise arises from the blepharoplasts (Fig. 26). The writer (1907), as well as Kofoid and Swezy (1915, 1915*a*), describes the axostyle as splitting longitudinally during division of *Trichomonas muris* and other species. Dobell (1909) stated that the new axostyles in *T. batrachorum* are formed from the two halves of the divided paradesmose, which connects the daughter blepharoplasts during division. Kuczynski (1914, 1918) claims that the old axostyle degenerates, and that new ones are formed as outgrowths from the daughter blepharoplasts, while the paradesmose disappears. Wenrich (1921) has described a similar origin for the new axostyles in *Trichomonas muris* (Figs. 271 and 272).

A great variety of fibres directly or indirectly connected with the blepharoplasts have been stated to occur in flagellates. Thus, Schaudinn (1904) describes numerous structures of this kind in *Trypanosoma noctuæ*, Prowazek (1903, 1904) in *Trypanosoma lewisi* and in *Herpetomonas muscarum*, while McCulloch (1915) figures a very complicated system of fibres in *Crithidia leptocoridis* (Fig. 154). It seems that the majority, if not all, of these are accidental structures, which cannot be considered as definite organs of the normal flagellates. Whether the marginal fibres of the cytostomal groove of *Chilomastix*, the basal fibre of the undulating membrane, and the axostyle of *Trichomonas*, and other similar structures which are connected with blepharoplasts, are to be homologized with

flagella cannot be considered as definitely established. It may also be open to question if the granules in which they originate, and which are generally styled blepharoplasts, are actually of this nature.

PHYSIOLOGY OF THE PROTOZOA.

Many of the physiological processes which regulate the life of Protozoa have been referred to above. It will only be necessary to review these in a general manner under the headings Nutrition, Movement, Reaction to Stimuli, Influence of Environment, Influence of Syngamy.

NUTRITION.—The essential food requirements of Protozoa are those of living matter in general. There is a constant expenditure of energy, necessitating a continuous supply of nourishment, which includes oxygen, simple chemical compounds, more complex organic substances, or highly organized proteid materials. Oxygen is an essential requirement, as it is of all living matter, but the method by which it is obtained varies, as it does between the vegetable and animal kingdoms. There are no special organs of respiration, so that absorption of oxygen and discharge of carbon dioxide takes place by a process of diffusion through the surface of the body. Certain Protozoa, like plants, possess chromatophores, and by means of their pigments or chromophyll are able, in the presence of sunlight, to obtain oxygen from the carbon dioxide which is in solution in the liquids in which they live, or which is formed by the organism itself. The chromatophores, which are green when they contain chlorophyll or red when the pigment is hæmatochrome, multiply by binary fission, as do also certain refringent granules called pyrenoids which they contain. They behave in many respects as independent organisms, and this has given rise to the view that they may be actually organisms living in a condition of symbiosis with the cells in which they occur. This method of nutrition is described as being *holophytic*, in contrast to the *holozoic* type, which is characteristic of Protozoa, which are devoid of chromatophores, and which must of necessity absorb oxygen directly from the liquid in which they live. In either case the organisms require oxygen, so that the two types of nutrition, the holophytic and holozoic, do not imply any essential difference in the character of the protoplasm of which their bodies are composed. This is well illustrated by certain species of *Euglena*, which normally have chromatophores, and lead a holophytic mode of existence (Fig. 6). Under certain conditions, as when cultivated in the dark with consequent loss of the pigment, they behave as organisms devoid of chromatophores. The holophytic forms nourish themselves like plants, and, in addition to the power conferred on them by the coloured pigments of being able to utilize carbon dioxide for the purpose of acquiring a supply

of oxygen, they are able to elaborate relatively simple chemical compounds into the protein materials necessary for their existence. Such forms may be cultivated in solutions of various salts, and, like plants, commonly elaborate starch or other amyloid substances as one of the products of assimilation, and not infrequently build for themselves capsules composed of cellulose. Between these and the completely holozoic forms, which require, in addition to oxygen, ready-formed proteid materials, either solid or in solution, there exists a group of organisms known as *saprophytes.* These do not possess chromatophores, but are able to live in fluids containing oxygen and complex organic compounds, which nevertheless are simpler than the proteid materials required by the truly holozoic types.

Amongst the holozoic Protozoa two methods of obtaining proteid material occur. In the one the organism ingests solid proteid material, mostly in the form of other living organisms, such as bacteria and other Protozoa, or, as in the case of parasitic forms like *Entamœba histolytica*, the cells of the host's body (Fig. 95). This solid matter is ingested either through a definite mouth opening or cytostome, or, when such is not present, through any part of the body surface by means of pseudopodia which surround it, or by a movement of the cytoplasm over the object, which appears to sink into its substance. In the other method, the proteid which is in solution is absorbed in liquid form. There is no mouth opening, the material merely passing into the body by osmosis. The latter method is characteristic of many parasitic Protozoa, such as trypanosomes, malarial parasites, coccidia, and gregarines. Other parasitic forms, such as the amœbæ, *Trichomonas* and *Balantidium*, ingest solid matter either by means of pseudopodia or definite cytostomes (Figs. 26 and 14).

In the case of Suctoria, which obtain their food by means of sucking tentacles, these are applied to solid objects, from which the proteid is extracted in a liquid form, probably as a result of ferments acting at the points of contact (Fig. 15).

As regards the proteid material ingested, two conditions result. When it is absorbed in solid form it is enclosed in food vacuoles, in which the particles are found in various stages of digestion (Fig. 70). When the proteid is absorbed in a state of solution no such food vacuoles are formed. From a study of the changes which occur during digestion in food vacuoles it has been found that when a living organism is ingested it is at first killed and then gradually digested, leaving finally a residuum of fæcal matter which is got rid of by the vacuole approaching the surface of the body and discharging its contents. In the ciliates there frequently exists a definite anal opening or cytopyge, usually at the posterior end of the organism, through which the residue is discharged (Fig. 512). The process of digestion is evidently the result of ferments which are secreted by the

cytoplasm, as various ferments have been extracted from Protozoa. Generally speaking, the reaction of a food vacuole is at first acid when the ingested organism is killed. The reaction then becomes alkaline. It is probable that during the acid phase a peptic ferment is active, while a tryptic ferment is present during the alkaline phase. Fats also are capable of being digested. It sometimes happens that the contents of a food vacuole are alkaline from the commencement, and it appears that the cytoplasm has some power of varying its response to different types of food.

The proteid material absorbed from the food vacuoles, or from the medium in which the organism is living, enters the cytoplasm, and is immediately elaborated into the constituents of the cell or leads to the formation of various intermediate bodies. The latter may be regarded as food-reserve materials which are merely accumulations resulting from the intake of excess of nourishment, or definite reserves intended for a period of excessive activity, such as occurs during the sporogony process of coccidia and gregarines, or the continued development when access to nourishment is prevented, as when a cyst wall is present.

In the organisms which have a holophytic method of nutrition the food reserve is stored largely as starch or allied substances of an amyloid nature. In gregarines preparing for sporogony in the gametocysts the cytoplasm becomes charged with refractile globules of a substance called paraglycogen. The macrogametocytes of coccidia, which are to continue development in an oöcyst, likewise become loaded with refractile globules of an albuminous substance. Similarly in the encysted stages of *Entamœba histolytica*, *Iodamœba bütschlii*, and other forms, a large amount of a glycogenic substance is present. It is gradually used up during the period passed by the encysted form in awaiting a suitable opportunity for emerging from the cyst. Another substance which is often present is volutin, which appears in the fresh condition as greenish refractile globules. It stains deeply with many nuclear stains, and has been supposed to be a forerunner of chromatin, but of this there is no direct evidence. Many of the granules which have been described as chromidia are probably of this nature. It commonly occurs in flagellates, and is often abundant in trypanosomes, appearing as deep red granules in specimens stained with Romanowsky stains. Fat globules also occur in Protozoa, and are commonly present in Radiolaria. The identification of the various granules and reserve substances is a very difficult matter, dependent on microchemical tests, solubility in various fluids, and reaction to different stains.

The residue from food digestion, as pointed out above, is discharged from the body. This may occur immediately after digestion is completed, or it may be deferred. The substances may assume different forms.

They may become crystalline excretory crystals, or remain as amorphous masses. Amongst the Sporozoa, when reproduction by schizogony takes place, a certain amount of cytoplasm is usually left over as a residual body, which takes no part in the formation of merozoites. In it is got rid of a certain amount of excretory substance. Malarial parasites thus discharge the pigment granules which accumulate as a result of digestion of hæmoglobin.

In addition to the substances which have been referred to, and which may be regarded as steps in the formation of protoplasm or the waste products from the food, there occur other substances which are elaborated to fulfil some special function. The conspicuous so-called chromidial body of shelled amœbæ may have to do with the formation of the shell. The various skeletal structures which occur in the cytoplasm of Radiolaria, the supporting rods which form the axes of the pseudopodia of many Heliozoa, and, indeed, the external coverings like the shells of Foraminifera and the cyst walls themselves, are to be regarded as products of metabolism. It is evident that the Protozoa which produce such structures must absorb special substances for the purpose.

Quite apart from the excretion of substances no longer required by the organism by the rupture of vacuoles containing them at the surface of the body, there is another method of excretion, which is carried out by a rhythmically contracting vacuole which is situated near the surface of the body. Such a contractile vacuole, when fully formed, suddenly contracts, so that the clear liquid contents are discharged through the surface of the body. In a short time the vacuole re-forms, and, gradually increasing in size, reaches its maximum, when it again contracts. In some cases definite channels in the cytoplasm conduct fluid to the vacuole. The rate of pulsation varies with temperature and the presence of substances which affect the density of the medium. It is supposed that the vacuole is a means of discharging carbon dioxide and other soluble excretory substances, but the fact that contractile vacuoles are absent in marine Protozoa and many parasitic forms, and that fresh-water forms lose the contractile vacuole when made to live in salt water, suggests that such a vacuole may be a means of accommodating the organism to the medium in which it lives, rather than an organ primarily excretory in function. It can hardly be supposed that marine or parasitic forms are less dependent on excretion for their existence than those which live in fresh water. It has been conjectured that the contractile vacuole may counteract the tendency of the cytoplasm to become overcharged with water due to the greater absorption in fresh than in saline water.

On the method of nutrition of any particular organism depends the character of the medium in which it can be cultivated. Forms like

Euglena, which possess chromatophores and behave like plants, can be grown in distilled water in which certain inorganic salts are dissolved. Saprophytic forms require more complex substances, while holozoic ones will grow only in media in which proteid material is present. This is usually in the form of bacteria, which form the staple food of amœbæ, flagellates, and ciliates, when grown on the surface of agar plates or in liquid media. In other cases, as in the cultures of trypanosomes and leishmania, bacteria are absent, the proteid materials being derived from blood-serum.

MOVEMENT.—The power of movement is one of the properties of cytoplasm in general, and amongst the Protozoa it is seen in its simplest form in organisms like amœbæ, and is most highly developed when special motile organs are present, such as flagella, cilia, the contractile filaments in the stalks of the attached Protozoa, and the myonemes of gregarines and other forms. The cytoplasm is in constant movement within the organism. This streaming of the cytoplasm is undoubtedly the result of chemico-physical changes which are taking place. In highly-organized Protozoa, like the ciliates, the currents in the cytoplasm are constant in their direction, and the various food vacuoles which move with them perform a definite circuit. In the amœbæ, which do not have definitely orientated bodies, there is more irregularity. It is as a result of this streaming of the cytoplasm that organisms like amœbæ are able to move and form pseudopodia. When resting on a surface, the portion of cytoplasm in contact with the surface is prevented from movement, while the streaming of the internal cytoplasm in one direction leads to a forward movement, which is best illustrated by the rolling movement of a bag of fluid on an inclined plane. In this manner the whole amœba may progress in one direction, or, when the streaming of the cytoplasm is limited, only portions will move forwards, with the result that pseudopodia are formed. By changes in the direction of the stream the pseudopodia are withdrawn and others protruded. Certain pseudopodia, like those of Heliozoa, are supported by axial fibres, which render them more permanent structures. They are, nevertheless, capable of performing swinging or bending movements. Whether these are the result of movements of the cytoplasmic covering or of the axial fibre has not been satisfactorily determined. As, however, fine pseudopodia devoid of axial fibres can perform such movements, it would seem that the axial fibre may be purely elastic in nature, with the function of bringing the pseudopodium back to its original extended position when the movements of the cytoplasmic covering cease. The more actively motile flagella and cilia of the Mastigophora and Ciliophora have essentially the same structure as the axopodia of Heliozoa. There is an axial fibre (axoneme) covered by a thin sheath of cytoplasm,

and it may be supposed that their movement is brought about in a similar manner by changes which occur in the thin cytoplasmic covering, the axial fibre acting as an elastic support. Similarly, the myonemes which occur in gregarines and other Protozoa may not in themselves be contractile, though they may limit the contraction of the cytoplasm itself to definite channels. It is generally supposed, however, that the filaments themselves are contractile. In the case of attached forms like *Vorticella* the stalk is composed of an axial fibre and a sheath of cytoplasm; when retraction takes place, the axial fibre assumes the form of a compressed spiral. During extension it appears that the elasticity of the axial fibre, which returns to its original condition, is responsible for the extension of the stalk, and it is possible that the sheath of cytoplasm is the sole cause of the retraction. That cytoplasm itself, quite apart from the presence of myonemes or other filaments, is able to perform sudden and rapid movements of contraction is illustrated by the behaviour of contractile vacuoles.

Another series of internal movements which are common to all cells provided with nuclei are those associated with nuclear division. The complicated process of mitosis, with the formation of the spindle and chromosomes, and the subsequent separation of daughter chromosomes, is in many cases carried out under the influence of the centrosome. In many Protozoa, however, no centrosome is visible, but in neither case has a satisfactory explanation of the phenomenon been given. When a centrosome is present, it appears to be the centre of activity, for it is towards it that the rays of the aster and the spindle fibres are directed. For those who regard the blepharoplasts of flagella as centrosomic in nature, the action of the flagella is supposed to be another illustration of the motor activities of the centrosome.

The movements of the cytoplasm which have been considered are distinct from the locomotion of the Protozoa themselves. An organism which is in a resting condition and undergoing no changes in shape may still show the streaming movement of the cytoplasm, but it is nevertheless these movements of the cytoplasm which bring about the changes in shape and actual locomotion when these occur. Progressive formation of pseudopodia and changes in shape in amœbæ are the result of continued streaming movements in one direction, as explained above. In the case of Mastigophora and Ciliophora it is the result of the continuous action of the special organs of locomotion, which are so arranged that when they are in activity the organism is propelled through the liquid medium. The peculiar gliding or slug-like progression of gregarines has been supposed to be due to the rapid secretion of a tenacious fluid from numerous pores in the longitudinal grooves of that portion of the ectoplasm which is in contact with the surface on which the organism is resting. It is

possible that the gliding movements performed by the small gregarine-like merozoites or sporozoites may be explained in a similar manner.

REACTION TO STIMULI.—The actual direction of progression is the direct result of external stimuli acting on the organism. Practically all Protozoa react to stimuli, whether mechanical, chemical, thermal, electric, or photic. The response to such stimuli has been chiefly studied in the case of ciliates, in which it has been frequently found that the region of the cytostome is the most sensitive part of the body. It is evident that for any Protozoon there is an optimum condition of the medium in which it lives, and if, during progression, it reaches an environment which is less favourable to its existence than that which it has just left, there will be a stimulation of the sensitive area of the body. This stimulation will result in an altered action of the organs of locomotion, with a consequent withdrawal from the unfavourable stimulus. The movements of ciliates when subject to adverse stimuli are very precise, and have been the subject of extensive investigations. The attraction and repulsion are known as positive and negative taxis respectively. Generally speaking, positive taxis indicates a movement towards and a negative taxis a movement from any particular environment.

INFLUENCE OF ENVIRONMENT.—The actual condition of the environment in which a Protozoon finds itself is a very important factor in its development. As already remarked, for each there is an optimum condition which suits it best. Departures from this are followed by conditions of depression resulting in degeneration or even death. Lack of food or excess of it, leading to starvation or overfeeding, also brings about degenerative changes which are seen in alterations in the structure of the nuclei, which frequently become enlarged. In certain cases the nuclei break up entirely, leading to the final death of the organism. To a certain extent Protozoa can be gradually adapted to changes in environment, provided these are not brought about too suddenly. It is possible by gradually raising the temperature of cultures to obtain a race of organisms which can live at a temperature which would have quickly killed if applied suddenly. Provided that degeneration has not proceeded too far, recovery is possible if the conditions are improved. Regeneration of the degenerate parts takes place. Similarly, Protozoa which have been mutilated or deprived of portions of their bodies are able to regenerate themselves, provided the nucleus remains intact.

The majority of Protozoa are able to protect themselves against adverse conditions by the process of encystment. The tough resistant capsule which is secreted shuts them off from their environment, so that they are able to survive unharmed till conditions favourable to a free-living

existence recur. Within the cysts the organism either undergoes no change or it may continue to multiply. In parasitic forms the cyst protects the organism during its passage from one host to another, the encysted form being known as the infective stage. Amongst the Sporozoa it usually happens that a period of asexual reproduction is followed by one in which sexual forms are developed. The appearance of these is generally supposed to be an indication that unfavourable changes are taking place in the environment, and that encystment, which occurs in association with conjugation and the production of the zygote, is necessary.

Another feature characteristic of many parasitic forms is the difference in environment associated with different stages of development. Thus, in the case of malarial parasites the human blood supplies the conditions necessary for asexual reproduction and the production of gametocytes. In the body of the mosquito all asexual stages quickly perish, while the gametocytes continue their development, which was arrested in the human blood. The sporozoites ultimately produced will develop no further in the mosquito, but with the change brought about by their injection into man further progress occurs. Similarly in the case of trypanosomes the forms taken up from the blood by the transmitting host quickly lose their power of developing in the blood, though they do so in the body of the invertebrate. The metacyclic trypanosomes which are eventually produced have regained the power of development in the blood.

As a result of abundance of nourishment in the medium the cytoplasm may become charged with globules of food-reserve material which appear to be far in excess of that actually required. Thus, the ciliate *Balantidium coli* may be packed with such substances. In many cases this has apparently little effect on the vitality of the organism, though it has been shown that in certain forms degenerative changes result.

A feature of this over-nourishment may be seen in certain cases of gigantism. Thus *Trichomonas vaginalis* is often very much larger than *Trichomonas hominis* of the intestine. If, however, both these organisms are cultivated in the same medium, the forms which appear are exactly alike, so that it would seem that the large size of *T. vaginalis* is merely an indication of overgrowth. Similarly the giant forms of *Herpetomonas mirabilis*, which occur in the Malpighian tubes of certain flies, can probably be accounted for in similar manner.

INFLUENCE OF SYNGAMY.—As already remarked, Protozoa which become degenerate or pass into a state of depression may recover if conditions of life become favourable. It is supposed that a similar recovery may result from the process of syngamy. In the majority of Protozoa, however, syngamy is not known to occur. In many cases this is undoubtedly due to the fact that the complete life-history has not

been elucidated. In some instances, however, unless it is assumed that syngamy must of necessity take place from time to time, it appears that reproduction by simple binary fission is continued indefinitely. Such an organism divides into two daughter individuals, and when these have become fully grown, division again takes place. A simple life-cycle of this kind is characteristic of the amœbæ, and it is only interrupted by the amœbæ becoming encysted under certain circumstances.

Within these cysts, which are purely protective in function, the amœbæ may or may not continue to multiply by fission. When conditions again become favourable, the cyst is ruptured and the amœbæ escape to continue their multiplicative existence. Similarly, many trypanosomes can be handed on indefinitely from one animal to another by simple inoculation of infected blood. There appears to be a continuous process of reproduction by binary fission without the intervention of either syngamy or encystment. Under natural conditions, however, direct transference from vertebrate to vertebrate, except in the case of *Trypanosoma equiperdum*, does not occur, the life-history being varied by alternate multiplication in a vertebrate and an invertebrate. As far as is known at present, multiplication in both hosts is by continuous binary fission, though some authorities assume that a syngamic process will be found to occur in the invertebrate. When such a change of hosts is obligatory, the parasite is said to require an *alternation of hosts* for the continuance of its life-cycle. In the case of certain blood-inhabiting Sporozoa (malarial parasites) the alternation of hosts is characterized by the occurrence of asexual multiplication in the vertebrate and syngamy followed by the production of sporozoites in the invertebrate.

Until recently it was considered that the periodic occurrence of syngamy was essential for the continued existence of the race. This view was the outcome of researches conducted on ciliates by Maupas and Richard Hertwig. Thus it was shown that *Paramecium caudatum*, after a varying period of multiplication by fission, proceeded to conjugate. Calkins (1904) found that, if conjugation was prevented, the ciliates, though they continued to reproduce, gradually weakened and died. Similar results had previously been obtained by Maupas (1888, 1889) in the case of *Stylonychia pustulata* and other forms. It was believed that these experiments proved that a race would invariably die out if conjugation did not occur. Enriques (1903), working with *Glaucoma scintillans* and *G. pyriformis*, and Woodruff (1917) with *Paramecium aurelia*, proved that this was not the case. The latter observer (1925), having commenced with a single individual, has carried on the culture by separating the daughter individuals produced at each division for a period of fifteen years, during which over 10,000 divisions have taken

place. Great care was taken to keep the culture medium favourable, and it was found that the ciliates were just as vigorous at the end of this period as was the original parent. It is thus evident that, even in the case of an organism which under natural conditions conjugates from time to time, the race may survive and still remain in vigorous condition when this is prevented. Woodruff (1921) showed that during this period of repeated binary fission the process of renewal of the macronucleus from the micronucleus, known as endomixis, took place at intervals (see p. 54). In the case of the ciliate *Spathidium spathula*, Woodruff and Moore (1924) have demonstrated that reproduction can be continued indefinitely without recourse to endomixis or conjugation when suitable environmental conditions are supplied.

From the work of Richard Hertwig and Maupas, who considered that conjugation was essential to survival of the race, arose the theory of rejuvenescence, which supposes that any race of ciliates dies out through loss of vigour if conjugation does not take place. It has generally been assumed that both these observers thought that the rejuvenating process showed itself in an increase in the rate of multiplication. According to Jennings (1920) this is a misrepresentation of their views, for it was definitely stated that the rate of fission before and after conjugation was not altered. Their view of the change which takes place in conjugation is that the ciliates which would otherwise have died now continue to live, and this continued existence itself is a sign of rejuvenescence. Calkins (1919*a*), however, has definitely asserted that the failing energy and rate of multiplication of the pre-conjugation period is abolished by conjugation, and that in the post-conjugation period the rate of multiplication is increased. Quite recently Woodruff and Spencer (1924), working with *Spathidium spathula*, have clearly shown that conjugation actually does increase the rate of multiplication, and, furthermore, that on an average cultures made from forms which have conjugated outlive those from forms which have not, so that the chances of any particular line surviving are increased. Careful experiments have not only shown that conjugation is not necessary to continued existence, but appear to have demonstrated, in many cases, that following it there is actual depression as regards rate of division, likelihood of death, and in other respects. If conjugation does not lead to some change of this kind, it is extremely difficult to account for the process of syngamy at all. It appears to be unnecessary, yet it takes place in nature. Minchin (1912) expressed the opinion that it tends to level down individual variations and keeps the species true to type. The true explanation may, however, be the reverse of this, as Jennings (1920) has pointed out.

It has been explained above that there occurs a reduction in the number

of chromosomes in the nuclei of gametes, or in the two nuclei into which the zygote nucleus divides. In this reduction division the individuals of each pair of homologous chromosomes are separated, one of each pair going to each daughter nucleus. If, for instance, there are four pairs of homologous chromosomes grouped as A*a*, B*b*, C*c*, D*d*, at reduction division one of each pair passes to a daughter nucleus, so that the daughter nucleus may receive chromosomes in many possible combinations—ABCD, ABC*d*, AB*cd*, A*bcd*, *abcd*, A*b*CD, etc. In all, there may be sixteen different combinations. When syngamy occurs, any one of these groups in one gamete will unite with any one in the other gamete, so that the zygote nucleus containing eight chromosomes will have a still larger number of possible combinations, the actual number being eighty-one.

It has been abundantly demonstrated in the higher animals and plants that the hereditary characters are intimately bound up with the various chromosomes occurring in the nuclei of the gametes, so that it is clear that union of gametes with four chromosomes will give rise to eighty-one different combinations of hereditary characters. In ordinary division without conjugation all the chromosomes split longitudinally, and half of each chromosome passes to each daughter nucleus, so that the hereditary characters are more equally distributed to the daughter nuclei. On this account, Jennings (1920) sees that the progeny resulting from conjugation show a greater diversity of hereditary combinations than do the progeny arising from multiplication by fission. From the point of view of survival of the race, the diverse individuals resulting from conjugation will be more likely to provide at least some forms which will tolerate any new condition of the environment than are the more uniform individuals which result from continued asexual reproduction alone. The group of organisms which result from conjugation will be at a distinct advantage when compared with others when changes in environment take place.

LIFE-HISTORY OF PROTOZOA.

The life-history of a Protozoon is one of continued growth and reproduction, which may or may not be interrupted at intervals by a process of syngamy. When syngamy occurs, two ordinary individuals which do not appear to differ from those which have been dividing may copulate, as in *Copromonas*, or conjugate, as in *Paramecium*, after which reproduction is resumed. On the other hand, it may happen that certain young individuals which arise in the usual manner, and which do not appear to differ from others which are destined to develop into forms like the parent, become transformed into individuals of a special type. They are known as gametocytes, which, when fully grown, produce a number of gametes.

The latter unite in pairs to form the zygotes, which give rise to typical daughter individuals, known as sporozoites. These grow into adults, which reproduce repeatedly in the manner characteristic of the reproductive phase till certain of the progeny again become gametocytes. The various forms which occur during the multiplicative phase, which is known as *agamogony*, belong to the *asexual generation*, while the individuals themselves are *agamonts*. In contrast to these, the gametocytes and the gametes to which they give rise, the zygotes, and the sporozoites which are ultimately formed, belong to the *sexual generation*. The process of development from gametocyte to sporozoite is known as *sporogony*, while the gametocytes themselves are *sporonts*. These two phases of development alternate in that, after reproduction has been repeated a number of times (agamogony), the sexual method of multiplication (sporogony) supervenes. The sequence of the two phases is known as *alternation of generations*, which is a characteristic of the majority of the Sporozoa. Amongst the typical gregarines, however, the asexual generation and agamogony does not occur, the sporozoites into which the zygotes divide growing directly into gametocytes, which again produce gametes. The whole life-cycle of a typical gregarine is thus one of sporogony.

As already indicated, the life-cycle of a Protozoon may at any time be interrupted by the formation of protective cysts secreted from the ectoplasm. Some organisms cease multiplying when they become encysted, others continue to multiply within the cyst, while others again never reproduce except in the encysted condition. Sometimes, as in the case of parasitic amœbæ, special individuals (precystic amœbæ) alone are capable of forming cysts. Amongst the Sporozoa, encystment only occurs in association with sporogony. A cyst may be formed around two gametocytes, as in the case of gregarines. It is then distinguished as a *gametocyst*. After syngamy has taken place, the resulting zygote may secrete a cyst known as an *oöcyst*. The zygote may divide into a number of sporoblasts, and these, either within the oöcyst or after their escape from it, become enclosed in secondary cysts called *sporocysts*. Oöcysts and sporocysts occur typically amongst the Sporozoa. The cysts usually have very tough and resistant walls; at other times they are little more than thin membranes.

Protozoa may be free-living organisms which spend the whole of their life in water or in moist situations, or they may be more or less intimately associated with other animals. According to the degree of this dependence three classes are usually recognized. There are *commensals*, which live in or upon another organism, and, though deriving benefit from this association, do not injure the host in any way. They deprive it of an

inappreciable amount of material which it might use itself, or feed upon the waste products. Others are regarded as *symbionts*, which, living in similar circumstances, not only derive benefit themselves, but contribute to the well-being of the host. Thus, Cleveland (1923) has shown that termites, which feed upon wood, do so by virtue of their intestinal Protozoal fauna, which actually digest the wood to form substances on which the life of the termites depends. Other forms are *parasites*, which deprive their hosts of their own fluids or tissues, and damage them by destruction of tissues either directly or indirectly through the formation of toxins. The line of demarcation between these various types is very indefinite, so that it is often impossible to decide to which group any particular organism belongs. The numerous discussions which have arisen as to the pathogenicity of the intestinal flagellates of man is a case in point.

When true parasitism is considered, it must be remembered that the degree of harm inflicted on the host has a direct bearing on the continued existence of the parasite. A parasite is an organism which has become adapted to an existence in another, and has lost at the same time the power of living outside this host. At some period of its existence it must be transferred to a new host if it is to survive. This transference may take place by the production of encysted forms which escape from the body and are taken up casually by a new host, or an invertebrate may take up the parasites from the blood and later introduce them to new hosts. In the first case the parasite does not appear to be able to produce the encysted stages till some time after infection of a new host has taken place, and in the second a period must elapse before the appearance in the blood of the forms capable of infecting the invertebrate. In any case, the chance of a parasite gaining access to a new host is a precarious one, and it is evident that the longer a parasite can survive in one host, the better is its chance of bringing about infection of another. If, then, a parasite is so virulent that it very quickly destroys its host, its chances of continued existence are definitely diminished. It is found in nature that there is such an adaptation of parasite to host, and *vice versa* that in all cases of parasitism the parasite damages its host to the least extent compatible with its own continued existence. Whenever a parasite is discovered which brings about the death of its host in a short time, it may safely be assumed that the host is not the natural one, or that it is a natural one which is in some unnatural condition. In the case of the pathogenic trypanosomes of Africa, the natural hosts are the antelopes, to which they do comparatively little harm, while human beings and domestic animals are unnatural hosts, as they are much more seriously affected. After a time adaptation may occur, and a host which was at first an unnatural one may gradually become a natural host.

Man seems already to have become a natural host to *Trypanosoma gambiense*, but to be only in process of becoming so for *T. brucei* (*T. rhodesiense*).

An important feature of parasitism is the specificity of any particular parasite for its host. It is found in nature that some parasites are unable to live in any other host than the one in which they naturally occur. This undoubtedly depends upon the peculiar character of the body fluids of these animals. Some parasites have become so specialized that they cannot survive in any other fluid than the one to which they have become accustomed. Very frequently, however, a particular parasite is able to live in hosts which are nearly related, the fluids of which may be presumed to differ only slightly from one another. Thus *Plasmodium vivax*, which causes benign tertian malaria, cannot survive in any other vertebrate host than man, though Mesnil and Roubaud (1920) have shown that it may multiply for a short period in the chimpanzee. Other parasites are much less specific, for many of the pathogenic trypanosomes can develop in small rodents, which undeı natural conditions are never infected by them. In such cases it seems probable that, quite apart from the suitability of the fluid of a host, the rapidity with which a host can develop antibodies is the determining factor as to whether a parasite can establish itself or not. Instances are known in which it is only after many attempts to introduce a parasite into a host that success is at last attained. An instance of this is quoted below (p. 576), where Watson, attempting to isolate a strain of *Trypanosoma equiperdum* from horses in laboratory animals, only succeeded in one after inoculating over 600 animals. The infection, once established, was then readily inoculated from one animal to another. It is evident that here the fluids of the animal which gave a successful result differed from those in which inoculation had failed, or that amongst the organisms injected on the successful occasion there happened to be a few which found the environment congenial and were able to resist the antibodies developed. The fact that subsequent subinoculations were easily carried out seems to suggest that the explanation is to be found in the parasites themselves. Not infrequently an animal which has acquired an infection will free itself, after which it is found to be immune to further inoculations. On the other hand, it has been shown that in some cases, when an infection has disappeared or has been much reduced, further inoculations of the same organism may bring about a superimposed infection which may be more severe than that first produced. Such an instance has been described by Nöller (1917) in the case of frogs infected with *Trypanosoma rotatorium*.

It may be stated as a general rule that the specificity of parasitic Protozoa for their particular hosts is much more marked than is the case

with vegetable parasites, such as bacteria, yeasts, and allied organisms. It often happens that a parasite in one host may be morphologically indistinguishable from one in another, yet experimentally it is impossible to produce cross-infections. Whether such biological races are to be regarded as distinct species or not is a problem which still requires solution. From the strictly zoological point of view they should be regarded as belonging to one. This highly developed specificity of Protozoan parasites may be kept in mind when organisms of a doubtful nature are being dealt with. The group of parasites known as *Toxoplasma*, which most observers regard as Protozoa, may actually be vegetable organisms, for it has been found that they are inoculable into a variety of different hosts.

Another feature exhibited by parasites is one which is termed increase in virulence. Here, again, illustrations occur amongst the trypanosomes. *T. gambiense* can be inoculated from man to laboratory animals. In the first passage the infection may be of slow development, but with successive passages through these animals a strain will develop which in its behaviour differs from that originally introduced. Whereas at first it may have taken a year to kill the animal in which the trypanosomes were always scanty, finally it brings about a fatal issue in two or three weeks, the trypanosomes reproducing rapidly till the blood of the animal is teeming with them. It is evident that during successive passages the trypanosomes have gradually adapted themselves to these animals. In the case of naturally occurring infections, which are characterized normally by a balance between host and parasite, occasionally infections occur in which such a balance does not exist. In naturally occurring malarial infections amongst native children exposed to the bites of infected mosquitoes there is a balance between the host and parasite, so that the host appears to be little inconvenienced. Sometimes, however, severe and fatal cases occur, either because the natural resistance of the host is low or because the parasites have become peculiarly virulent. These severe infections are of more frequent occurrence amongst human beings who have come from non-malarial countries and are suddenly exposed to infection. It is often claimed that these cases result from a specially virulent strain of parasite, but it seems more probable that the host is at fault, and that the fluids of the body differ from those of the natural hosts. Another illustration is seen in the case of *Entamœba histolytica*. In the majority of cases of infection with this amœba, the organism produces a minimum of inconvenience to its host, which is known as a carrier, but in a small percentage of cases the balance is broken down and acute symptoms of amœbic dysentery reveal themselves.

It is found that the reaction of a host varies with the strain or race of

any particular parasite employed. Two strains of the same species of trypanosome may produce very different results. An animal inoculated with one strain may acquire an infection from which it will recover. It may have developed an immunity and be no longer inoculable with this particular strain, though it is still susceptible to inoculation with another strain of the same species. On this account it is exceedingly difficult to differentiate species of trypanosome by what have been termed immunity experiments.

The mechanism of these various phenomena are far from being properly understood, and it appears that a real explanation will never be obtained till the biochemist has obtained more information regarding the chemistry of the living cell and the fluids to which it gives rise.

IMMUNITY IN PROTOZOAL INFECTIONS.

Immunity in connection with parasitism amongst the Protozoa will be referred to below in connection with individual parasites, but it will be necessary to discuss more fully some of the general features which have just been mentioned above.

NATURAL IMMUNITY.—As remarked above, each parasite has its own particular host or group of hosts in which it can live, and outside these limits it is impossible for it to establish itself. This specificity, as it is called, is well illustrated by the malarial parasites of man. Exactly how infections are prevented in one host while they take place readily in another is not properly understood, but, as a result of extensive researches, it is evident that cells and fluids of the body of refractory animals are of such a nature that parasites introduced cannot develop and are finally killed. That the serum of the blood is largely responsible for this natural resistance is proved by the experiments of Laveran (1904*a*), who showed that the blood-serum of baboons, which are usually refractory to inoculation with *Trypanosoma gambiense*, when injected into mice will cause the disappearance of *T. gambiense* from their blood, or even prevent infection if injected forty-eight hours before inoculation with the trypanosome. Such an immunity against infection is a natural immunity. It is possible, however, in some cases to overcome the natural resistance. This may be effected either by lowering the resistance of the inoculated animal, an illustration of the well-known fact that a person in good health is less liable to disease than one who is in poor condition, or by increasing the virulence of the parasite. As a rule mice and guinea-pigs are quite refractory to inoculations with *Trypanosoma lewisi* of the rat, but Roudsky (1910*a*, 1911), as will be mentioned below, was able to increase the virulence of the trypanosome, so that mice and guinea-pigs were susceptible.

It is thus evident that in a study of the interrelations of a host and the parasite both the condition of the host and that of the parasite have to be taken into account. The increase in virulence of *Trypanosoma lewisi* produced by Roudsky was artificial, and it is probable that under natural methods of transmission such a change would rarely, if ever, take place. Nevertheless, the observation is an important one, for it demonstrates that a trypanosome may become modified to such an extent that it will produce infections in animals in which normally it fails to develop. It is a generally accepted fact that the animal trypanosome, *Trypanosoma brucei*, does not as a rule infect man who is constantly exposed to the bites of infected tsetse flies, yet there occurs in man in Rhodesia a trypanosome which has been given the name *Trypanosoma rhodesiense*, which in all respects appears to be identical with *T. brucei*. It is maintained by some that it is distinct from *T. brucei*, and by others that it is identical with it. It has, however, to be recognized that it is quite within the bounds of possibility that the animal trypanosome *T. brucei* may occasionally change, for reasons not yet discovered, so that it becomes capable of infecting man, or that man may occasionally be in a condition which will permit infection with the unaltered trypanosome. Duke (1923, 1923*a*) believes that an outbreak of trypanosomiasis amongst human beings in the Mwanza district of Africa, in which the trypanosome was of the *T. rhodesiense* type, was due to the inoculation of the animal trypanosome *T. brucei* as a result of the lowered resistance of the population after a period of famine and heavy ankylostome infection.

There are many examples of variation in virulence of parasitic Protozoa. It is well known that if *Trypanosoma gambiense* is inoculated from the blood of man into a rat, the type of infection produced is a chronic one, very few trypanosomes being present in the blood of the rat at any one time, the inoculated animal often surviving for many months. In successive passages in rats the virulence increases, till finally a strain is produced which multiplies very rapidly, so that the blood is soon swarming with parasites, which bring about the death of the host in about ten days. By passage of the strain through a different host such as the guinea-pig this virulence for rats may be largely lost. It is regained, however, by further passage through the rat. Duke maintains that in the spread of sleeping sickness the epidemic outbursts of this disease are due to direct passage of the trypanosome from man to man by mechanical transmission in which some biting insect merely conveys blood from an infected to a healthy person, just as in laboratory experiments the syringe conveys blood from an infected to a healthy animal. It is supposed that in this way the virulence of the trypanosome, which is kept relatively avirulent

under ordinary conditions by a definite cyclical development in the tsetse fly, is greatly increased.

It is noteworthy that Blanchard and Blatin (1907) have shown that the marmot during hibernation at a temperature of 6° C. becomes resistant to trypanosomes, with which it can readily be inoculated when it is in an active condition. Brumpt (1908*a*) found that the dormouse showed a similar immunity during hibernation, though it was observed that the trypanosome (*T. blanchardi*) with which it may be naturally infected persists in its blood during the hibernation period. The natural susceptibility or the resistance of animals to infection with parasites has been advocated as a means of differentiating species. The method has been mostly used in the case of trypanosomes, but it has been also applied to other parasitic Protozoa. As an example may be quoted the effect of inoculating into rats the two trypanosomes *T. congolense* and *T. nanum*, which in their natural hosts are morphologically indistinguishable from one another. When inoculated into rats *T. congolense* gives rise to an infection, while *T. nanum* does not, and it is claimed by the advocates of the specific value of this test that the difference justifies the separation of the two species. That the test is not as straightforward as at first it might appear is illustrated by the fact that if *T. congolense* is inoculated into a goat, it will be found to have lost its power of infecting rats. It follows, therefore, that distinction of species based solely on the ground of resistance of certain animals is zoologically unsound. Another application of the same test was made by Adler (1924), who discovered a coccidium in the intestine of the civet cat in West Africa. Morphologically it resembled *Isospora rivolta*, a parasite of dogs and cats. Attempts to infect dogs and cats with the parasite of the civet cat having failed, it was thought justifiable to establish a new species. Looking at the question from the reverse point of view, the susceptibility of a number of different hosts to a parasite derived from one host is strongly suggestive of the identity of the parasites which may occur naturally in a variety of hosts. Thus, birds are very liable to natural infection with a malarial parasite, *Plasmodium præcox*. The demonstration that the parasites from one bird can be inoculated into birds belonging to other species is a valuable indication that the one parasite may, under natural conditions, occur in a variety of hosts. The converse is not necessarily true, for development in one host may bring about such a change in the parasite that it is no longer able to infect a host which was originally susceptible to it. The example of passage of *Trypanosoma congolense* through the goat, referred to above, is a case in point.

In connection with natural immunity it has to be remembered that much depends upon the number of parasites—the dose of virus—intro-

duced. Theoretically it would be expected that in the case of susceptible hosts the introduction of a single parasite would bring about infection. This has actually been demonstrated in the case of *Trypanosoma brucei* and mice by Oehler (1913), who showed that the introduction into the peritoneal cavity of a single trypanosome gave rise to infection. In other cases, as, for instance, in the inoculation of *Leishmania donovani* to animals, no infection can be detected unless comparatively large doses are employed. In animals with absolute immunity no infection occurs even after the use of massive doses. Experiments such as these have been conducted with animals which are not natural hosts of the parasites concerned, but there is evidence that even in the case of natural hosts infection does not always follow exposure, a result which may depend on the dose of the virus.

Even when a host is a natural one there are always certain individuals which resist infection. It is well known that, though human beings are very susceptible to malaria, there are certain individuals who appear to have a natural immunity, and never show any evidence of infection, though constantly exposed to the bites of infective mosquitoes. Mühlens and Kirschbaum (1924), during the inoculation of human beings with malaria, observed one case which proved resistant to four inoculations, but became infected after a fifth.

RECOVERY FROM INFECTIONS.—It is a general rule that when once a parasite has established itself in a host it multiplies actively for some time, so that the intensity of the infection rises to a maximum, after which it gradually subsides till finally there may be every reason to suppose that the infection has completely died out. This recovery may be due to two causes. Firstly, the fluids of the host may gradually change with the production of substances injurious to the parasite, or possibly by the loss of substances which are necessary to the continued development of the parasite; secondly, the parasite itself may become exhausted and no longer capable of multiplication unless some change takes place. In the case of coccidial infections of animals, during the early stages there is active multiplication by schizogony in the intestinal epithelium. Gradually this multiplication subsides, and there are produced an increasing number of male and female gametocytes, which lead to syngamy and the formation of oöcysts, which leave the body. Eventually the sexually differentiated forms alone can be found, and finally the infection ceases when all these have been eliminated. In this case it is possible that the host produces substances which act deleteriously on the parasite, and lead to the production of the sexual stages, which are bound up, in the case of the coccidia and other forms, with the distribution of the parasite to other hosts. On the other hand, it may be that each sporozoite freshly

introduced is only capable of reproducing asexually a certain number of times, and that when this is completed sexual forms are produced. It seems clear that the production of substances which are generally termed antibodies in the blood of the host plays some part, for when once a host has passed through an acute infection it is rarely possible to produce as intense infection again, while in many cases a complete immunity to further infection is developed. But the second factor also comes into play, for it has been shown that as one infection is subsiding it may be possible to reinoculate the host with the same organism, so as to produce a superimposed infection. Nöller (1917) has shown that frogs which have passed through the acute stage of an infection with *Trypanosoma rotatorium* may be reinfected, though trypanosomes remaining from the first infection are still present in the blood in small numbers. Such a superimposed infection may become as intense as the first one, and even bring about the death of the host. Similarly in the case of piroplasmosis of cattle, Ed. Sergent and his co-workers (1924) have demonstrated that superimposed infections are possible. They found that the appearance of parasites in the blood after the second inoculation was not accompanied by any of the symptoms which followed the first infection. The animals had been rendered partially immune, so that the injurious effects of the parasite were resisted, though its development was not prevented. In order to distinguish this partial immunity or tolerance immunity from an absolute or true immunity they have introduced the term "prémunition." It occurs in the infections with *Babesia bigemina*. The term is not applicable to infections with *Babesia mutans*, which can also be superimposed on an already existing infection, for the first infection is not accompanied by any recognizable symptoms. This parasite appears to produce no immunity whatever. Hoare (1923) found that sheep, when constantly infested with keds, always harbour *Trypanosoma melophagium*, but if the animals are freed from keds the infection in the sheep gradually subsides, till after two or three months it can no longer be detected. It is evident that the batch of parasites introduced by the keds on one occasion have only a limited term of existence in the sheep, and it would appear that this is dependent rather on what may be termed an exhaustion of the parasite than on changes in the sheep, for infection may at any time be re-established by further introduction of trypanosomes from the keds. This exhaustion, however, may be the result of continued action of the antibodies producing a gradual weakening of the parasite.

It seems clear that in the case of many human Protozoal infections, such as malaria, trypanosomiasis, and amœbiasis, in localities in which these diseases are prevalent, individuals are constantly being infected with fresh batches of parasites, and a condition resembling that in the

sheep, just mentioned, occurs. In malarious countries, from their birth children are constantly being bitten by infected mosquitoes, and it is not unreasonable to suppose that the long duration of malarial infection in children in these countries is due to continuous reinfection. It has been demonstrated by Mühlens and Kirschbaum (1924) that human beings can be reinoculated with malaria when apparent recovery from a first infection has taken place. They can even be inoculated a third time, but the successive infections are of decreasing intensity. In view of the difficulty in determining the complete elimination of parasites from infected individuals, it is possible that some of these cases were illustrations of superimposed infections. Recently Van Loon and Kirschner (1924) in the Dutch East Indies have noted that the native is relatively immune to inoculation of malarial parasites. In certain cases it was found to be impossible to produce infection, though large doses of blood heavily infected with *Plasmodium vivax* were injected four or five times. In other persons who had not experienced a lifelong exposure infection was readily produced. Sergent, Et. and Ed. (1921*c*), have, however, shown that birds in the chronic phase of a malarial infection do not respond, or respond very slightly, to inoculations with a further infective dose of parasites. A very striking illustration of the effect of repeated doses of a virus was an observation made by Miller (1908) on the hæmogregarine *Hepatozoon muris* of rats. As a rule these animals, which are infected by the ingestion of mites, acquire an infection which does not appear to disturb the host in any way. Miller, however, found that a batch of rats, which were so heavily infested with mites that constant infection with large doses of virus was occurring, were very heavily infected with the parasite, and that a definite pathological condition resulted. When recovery from an infection is considered, a distinction has to be drawn between the cases which have had a single dose of virus and those which are repeatedly inoculated. Though recovery in a comparatively short time appears to be characteristic of many Protozoal infections, this is not invariably the case. Animals such as cattle, horses, and dogs, which are liable to piroplasmosis, pass through an acute phase when parasites are exceedingly numerous in the blood. Afterwards the infection subsides, so that finally the organisms can no longer be detected by microscopical examination of the blood. Nevertheless, it can be demonstrated that they are still present and persist for years, by the inoculation of large quantities of blood into animals which have never had the infection. In many cases of infection with *Entamœba histolytica* the amœbæ persist in the intestine indefinitely. In these cases a balance between the host and parasite has been reached, so that the former is injured to a minimal extent, while the parasite can reproduce sufficiently

to maintain itself. Hosts in this condition are usually termed carriers. The practical difficulty associated with this type of infection is the impossibility of being absolutely certain that any infection has entirely vanished. In the treatment of trypanosomiasis, leishmaniasis, malaria, amœbic dysentery, and other infections, this difficulty is constantly being encountered.

Another feature of recovery from infection has to be noted, and that is that frequently during the period of abatement of the infection, when the host may be said to be obtaining a mastery over the parasite, a relapse occurs in which a fresh outburst of activity on the part of the parasite leads again to an intense infection. It must be supposed that under these conditions the control of the host over the parasite has broken down, and anything which leads to this may bring about a relapse. It is well known that in malarial infections of man a sudden exposure to cold, shock resulting from accident, or the intercurrence of some other infection, may lead to the appearance of large numbers of parasites in the blood.

Such periodic variations in the intensity of infections may, however, be a feature of the development of the parasite. This periodicity is quite distinct from the periodicity which results from the developmental cycle, like that of parasites of malaria, which reproduce only at regular intervals. In human trypanosomiasis, and also in animals experimentally infected, it has been frequently noted that the number of parasites in the blood is not constant. The trypanosomes may be comparatively numerous on one day and absent on another. This is probably due to variations in the rate of multiplication, but it is possible that it is also dependent on variations in the rate of mortality of the trypanosomes resulting from irregularities in the antibody content of the body fluids of the host. No satisfactory explanation of this type of periodicity has been discovered.

ACQUIRED IMMUNITY.—Under this heading will be considered the immunity to infection which a host acquires as a result of an infection. It has already been shown that in some cases infection may persist for many years in a latent form, and though there may be considerable difficulty in determining the complete elimination of an infection, there is reason to suppose that sometimes a host becomes completely free. After recovery of this kind the host may be absolutely immune to further infection, the type of immunity being known as active immunity. The observations of Van Loon and Kirschner, who failed to produce malarial infections in natives of the Dutch East Indies, have been referred to above. In human infections with *Leishmania tropica* the disease oriental sore, if allowed to run a natural course, will produce in most cases an absolute immunity to further infection, so much so that artificial production of oriental sore by inoculation on an unexposed part of the body has been

employed as a means of avoiding the risk of the disfiguring natural infection on an exposed part such as the face. Another illustration of absolute immunity conferred by a single infection occurs in the case of East Coast fever of cattle due to infection with *Theileria parva*. Animals which have recovered from one attack are immune for the rest of life. The same remark applies to rats which have recovered from an infection with *Trypanosoma lewisi*. Again, in the case of many of the disease-producing trypanosomes it has been found that certain animals, such as the goat and sheep, though acquiring an infection, eventually recover to such an extent that trypanosomes can no longer be detected. In this condition they are immune to further inoculations with the same trypanosome. As in the case of naturally immune animals, these actively immunized hosts have been employed as a means of differentiating species. If it is desired to distinguish two trypanosomes which resemble one another morphologically, one of them is inoculated into a goat. When the animal has recovered and is no longer susceptible to inoculation with this trypanosome, it is inoculated with the other. If infection occurs, it is assumed that the trypanosomes are different. Though the experiment undoubtedly indicates a physiological difference between the trypanosomes, it is far from clear that they belong to distinct species. The test has been applied by Laveran and Mesnil and others to a group of trypanosomes which resemble *Trypanosoma evansi*, with the result that a number of species of very doubtful value has been created. Similarly, in the case of piroplasmosis the test has again been applied. Animals which recover from an acute attack pass into a chronic phase, during which the parasites show a gradual diminution in their numbers, till finally they can no longer be detected except by the inoculation of comparatively large quantities of blood into a susceptible animal. It has been shown by Ed. Sergent and his co-workers (1924) that in the case of *Babesia bigemina* it is possible to produce a superimposed infection in which parasites appear in the blood, but this is unaccompanied by symptoms. The infection, moreover, is less intense than the original one, the parasites quickly disappearing again. Stockmann and Wragg (1914) showed that cattle which had recovered from an infection with *B. bigemina*, and were immune to further inoculations with this parasite, were nevertheless susceptible to *Babesia bovis*, and behaved, as regards symptoms and intensity of infection, as animals at their first infection. In this instance there were morphological differences which justified the separation of the two parasites as distinct species. On the other hand, a form of piroplasmosis in cattle in South America is due to a parasite resembling *B. bovis*. Brumpt (1920) showed that cattle which had recovered from the infection with this parasite were still susceptible to inoculation with

the one from South America. There appear to be slight morphological differences between the two, but whether these are sufficiently distinct to justify the recognition of the South American form as a distinct species, *Babesia argentina*, apart from the cross-immunity test, is open to question. In connection with piroplasmosis of horses, Nuttall and Strickland (1910) and du Toit (1919) showed that animals recovered from infections with *Babesia caballi* were still liable to infection with *Babesia equi*. Here again morphological characters enable the species to be distinguished. The difficulty of accepting the test as a means of distinguishing species is illustrated by the experiments of Laveran and Nattan-Larrier (1913) on canine piroplasmosis. The disease occurs in dogs both in France and North Africa, and on morphological grounds appears to be due to the same parasite, *Babesia canis*, in both places. Yet dogs which have recovered from infection with the French virus and are completely immune to further inoculations are susceptible to the North African virus. It would appear impossible on these grounds alone to recognize two species of parasite.

As in the case of natural immunity, acquired immunity is dependent on antibodies which appear in the blood, for the serum of the animals which have recovered or have been infected for a length of time sufficient to allow of the production of these substances can be employed as a curative agent in the case of infected animals. Furthermore, the serum, when injected into an animal before it is exposed to infection, may entirely prevent an infection. In this case the immunity is known as passive immunity, because the host itself has taken no part in the production of the antibodies, which are merely introduced from another animal. The extensive investigations of Rabinowitsch and Kempner (1899), and of Laveran and Mesnil (1901*a*), on infections of rats due to *Trypanosoma lewisi* threw considerable light on this subject. Infected rats pass through an acute phase followed by a chronic one, from which ultimate recovery takes place. The animals are completely immune from reinfection. A small quantity of the serum (0·5 c.c.) of a recovered animal, if inoculated into the peritoneal cavity of a rat, will entirely prevent infection when trypanosomes are inoculated twenty-four hours later. This property is possessed, though to a less extent, by the serum of animals, such as goats and sheep, which have recovered from infections with the pathogenic trypanosomes, and animals, such as cattle, which are in a very chronic stage of infection. Taliaferro has shown, in the case of *T. lewisi*, that this is due to the appearance in the blood of the rat of a substance which inhibits the reproduction of the trypanosomes (see p. 467).

Many attempts to produce an active immunity by other means than actual infection and natural recovery have been made. So-called attenu-

ated strains, such as trypanosomes which, as a result of exposure to heat or other adverse conditions, have lost their power of producing actual infection, have been injected into animals. In a similar manner killed trypanosomes, trypanosomes which have been broken up by immersion in fluids which bring about cytolysis, dried trypanosomes, as well as cultural forms of trypanosomes, which often have ceased to be infective to animals, have been tried, but in none of these cases was satisfactory evidence obtained that the animals inoculated with these altered trypanosomes had acquired any immunity to inoculation with a virulent strain, though the application of certain serological tests, such as that of the complement fixation, has demonstrated that a specific change may have taken place in the serum of the animals. The response as regards production of immunity cannot be compared with that which occurs in the case of bacteria. Ponselle (1923*a*) has found that by keeping the heart-blood of a mouse containing *Trypanosoma brucei* for twenty-four hours in a medium of dihydrogen potassium phosphate and hydrogen disodium phosphate it loses its power of infecting mice, but if injected will render mice immune to infection with unaltered *Trypanosoma brucei* (see p. 454). The bulk of work in connection with the production of immunity in Protozoal infections has been carried out with trypanosomes, but certain investigations have been made with other Protozoa. Thus, the Sergents, Et. and Ed. (1921*b*), have produced a certain degree of immunity in the case of the parasite of bird malaria, *Plasmodium præcox*. Normal canaries were very easily infected with this parasite, only 0·72 per cent. resisting infection out of 965 birds inoculated. If canaries are inoculated with the sporozoites of the parasite which have been rendered non-infective by keeping them for twelve to forty-eight hours after removal from the mosquito, a certain degree of immunity results. It was found that 29·5 per cent. of twenty-four canaries thus treated resisted subsequent inoculation with the parasite. Similarly, it was found that if the blood of a canary was drawn off after it had been inoculated with the parasite, and before the infection had established itself by the appearance of parasites in the blood, this blood, if injected into healthy birds, produced an immunity which protected from subsequent inoculation 21·3 per cent. of sixty-one canaries.

Many observers have attempted to produce immunity in cattle against infection with *Babesia bigemina* and *Theileria parva*. From both these infections animals may recover naturally, and possess an absolute immunity to further infection, but the death-rate is always high, especially in the case of East Coast fever. No means of producing an immunity apart from actual infection are known, though in the case of piroplasmosis it is possible to inoculate the animals at a time when they are best able to

withstand the disease. It is known that young animals recover more easily than older ones, and that the disease is less severe at a certain season. It has been shown by a number of observers that by inoculating young animals with *Babesia bigemina* at this particular season it is possible to obtain a higher percentage of recoveries, and hence of permanently immune animals, than if they had been exposed to natural infection. In the case of East Coast fever also young animals are less seriously affected than older ones, and it would be expected that a similar method of protection could be applied. As will be shown below, it is not as a rule possible to transmit this disease by the inoculation of the blood of an infected animal, but Meyer (1909) found that this could be effected by inoculating the macerated spleen and lymphatic glands in which the reproducing forms occur. By the inoculation of young animals with emulsions of these organs Theiler (1911*a*, 1912*b*) noted that though a number acquired a severe and fatal disease, a much larger number survived and recovered completely. As many as 50 per cent. of those which survived proved resistant when exposed to infection by ticks under natural conditions. Somewhat similar results were obtained by Wölfel (1912) and Spreull (1914). In the production of immunity by these methods it is important, as demonstrated by Theiler (1908) and Lignières (1903), to employ the particular strain of virus to which subsequent exposure will occur. A previous infection with *Babesia bigemina* of European origin will not produce immunity against the parasite of South Africa.

Mechanism of Immunity.—During the development of an immunity the blood of the animal acquires certain properties which it did not previously have, but which are possessed by the blood of naturally immune animals. It has already been pointed out that the serum of such an animal will produce a degree of passive immunity when injected into a healthy animal, which is thereby protected against inoculation with the organism. Such passive immunity is usually of much shorter duration than active immunity, which is due to the production of antibodies by the host itself as a result of an actual infection, or the introduction of modified or dead parasites, or the products of their dissolution, which stimulate the host to produce the antibodies without actually giving rise to an infection. Where active immunity is produced without infection, the substance introduced is termed a vaccine. It is evident that the immunity produced is dependent upon the presence of several distinct substances, each of which has its special action. It was first shown by Laveran and Mesnil (1901*a*) that during the course of an infection with *Trypanosoma lewisi* the leucocytes of the rat's blood are constantly ingesting trypanosomes, which are ultimately destroyed. It appears that the serum of an immune

animal actually stimulates this phagocytosis, for Laveran and Mesnil found that if the serum of such an animal was mixed with trypanosomes and injected into the peritoneal cavity of a rat, there appeared in the peritoneal fluid numerous leucocytes which devoured the trypanosomes with avidity. If the trypanosomes were injected alone, this phenomenon was not observed to anything like the same extent. Levaditi and Muttermilch (1911) showed that the serum affected the trypanosomes in such a way that they attached themselves to the leucocytes. This was independent of the actual process of phagocytosis, for it was found that attachment to killed leucocytes also occurred. It was shown by Mesnil and Brimont (1909) that if immune serum were allowed to act upon *Trypanosoma lewisi* a change took place, so that the trypanosomes were no longer able to infect rats even if they were carefully washed free of serum. It would thus appear that the protective action of the serum is a result of its power of causing the trypanosomes to attach themselves to the leucocytes which then engulf them. The serum of animals which are immune to *Trypanosoma lewisi* also has the property of causing trypanosomes to become agglutinated into clumps when blood containing them is mixed with the serum (see p. 452). The presence of agglutinins in the serum has been shown to occur in the case of other trypanosome infections.

Another property which the serum may acquire is that of producing cytolysis, or the gradual swelling up and dissolution of trypanosomes exposed to its action. It was shown to occur in the case of infections of animals with the pathogenic trypanosomes by Levaditi and Muttermilch (1909), amongst other observers. They also demonstrated that the serum acquired the property of deviating the complement, a reaction which has found a practical application in the diagnosis of trypanosome infections (see p. 452). It seems evident that recovery from any infection is dependent on the development of antibodies in the blood, which act upon the particular parasites in various ways. This action of the serum of an immune animal is specific for the parasite which stimulated its production. On this account serological tests, which are similar to the inoculation tests referred to above, have been employed as a means of differentiating parasites. If the serum of an immunized animal behaves towards an unidentified trypanosome as it does towards the one which caused the immunity, then, provided that there is morphological similarity, it is concluded that they are identical. On the other hand, it is maintained by some that, in spite of morphological identity, if the serum fails to act it is proof of a specific distinction. It is possible that a natural recovery would never take place unless antibodies were produced, and that a parasite would continue to multiply continuously till the host

was destroyed. Certain strains of pathogenic trypanosomes can be handed on indefinitely from mouse to mouse by direct inoculation of blood without there being any evidence that the rate of multiplication by binary fission slackens in any way. In these cases the trypanosomes multiply so rapidly that the host is overcome by the parasite before any degree of immunity capable of checking the infection has been developed. At each inoculation the trypanosomes are introduced into a new host which has no immune bodies, and multiplication is continued with the same result. For the development of immunity it is essential that the rate of multiplication of a parasite shall not be so great as to bring about destruction of the host before it has time to respond to the infection by the production of sufficient antibodies to check the development of the parasite. From the point of view of the parasite this is the condition most favourable to its survival, and it appears to be the one which obtains in most, if not all, natural infections.

A parasite may acquire the power of resisting the antibodies in the serum. Jacoby (1909*a*) obtained a strain of *Trypanosoma brucei* which was resistant to human serum, which normally will cause the disappearance of the trypanosomes from the blood of mice. By repeatedly injecting small quantities of normal human serum into an infected mouse and continuing the process in subinoculated mice, a strain of trypanosomes was eventually secured which, as regards its development in mice, was uninfluenced by as large a dose (2 c.c.) of human serum as the mouse could tolerate. Lebœuf (1911) in a similar manner obtained races of *T. brucei* which were resistant to the serum of baboons.

ACTION OF DRUGS IN PROTOZOAL INFECTIONS.

It is possible that the disappearance of parasites as a result of the administration of drugs is, in many cases at least, not the result of a direct poisonous action of the drug upon the parasite. It would seem natural to suppose that the good effects observed in amœbic infections which result from the use of emetine and those following the ingestion of quinine in malaria are due to the direct effect of the drugs upon the parasites concerned. It appears that the action may be a much more complicated one, and that drugs may act indirectly by stimulating the tissues of the host to produce substances which may be regarded as antibodies which are directly responsible for the suppression of the infection. In support of this contention may be urged the fact that drugs such as emetine, which are therapeutically active, are not more toxic to the organisms when tested *in vitro* than other drugs which have no therapeutic properties. The investigations of Dale and Dobell (1917) on the

action of emetine are discussed below (p. 255). Morgenroth (1918) believes that quinine combines with the red blood-corpuscles, and thus prevents the entry into them of the merozoites of the malarial parasites. Quite recently Yorke and Macfie (1924*a*) have suggested that in malaria quinine acts by causing a destruction of a certain number of parasites, the broken-down parasites then acting as a vaccine in stimulating the host to produce antibodies, which finally rid the host of all remaining parasites. So far the presence of the antibodies has not been demonstrated. Another illustration of what may be the indirect action of a drug is seen in "Bayer 205." This medicament is remarkably trypanocidal when injected into animals infected with certain trypanosomes. Animals which have recovered as a result of treatment or uninfected animals which have received a dose of the drug remain immune from infection for comparatively long periods. It is possible that this resistance is due to the production by the host of antibodies as a result of the action of the drug upon its cells. On the other hand, it has to be remembered that when a drug is administered to an animal it does not follow that the drug remains unaltered. The fluids of the body act upon it chemically, and may in this way produce other substances which are definitely toxic to the parasites. It is known that arsenic compounds in which the arsenic is in the trivalent form are toxic to trypanosomes *in vitro*, and are also therapeutically active, whereas when the arsenic is in the pentavalent form there is no action *in vitro*, though there is a therapeutic action which, however, requires some time to develop. This difference has been explained by the fact that in the body of an animal the pentavalent arsenic radical is transformed into a trivalent one.

Another feature of the action of drugs on Protozoa is the development of drug-fast strains. In the case of mice, for instance, infected with pathogenic trypanosomes, the repeated treatment of the infection with such a drug as atoxyl in doses which are insufficient to prevent a subsequent relapse will finally result in a strain of trypanosome which is quite unaffected by the drug administered to the animals. This strain maintains its resistance when passed through a series of new mice, but as Mesnil and Brimont (1908 *b*) discovered, it is susceptible to the drug when inoculated into rats, and is still resistant when again passed into mice. Such a fact appears to be explicable only on the assumption that the trypanosomes have not become resistant to atoxyl itself, but to a substance resulting from the action of the drug on the tissues of the mouse, but not of the rat. Furthermore, it has been demonstrated that trypanosomes can be rendered arsenic resistant by the inoculation of infected mice with substances which contain no arsenic. Many writers refer to quinine-fast strains of malarial parasites and emetine-fast strains

of *Entamœba histolytica*, but at present there is no reliable evidence that these actually exist. A drug which fails to act on a parasite may do so because of some peculiarity on the part of the host. The whole subject of the method of action of drugs in the treatment of Protozoal infections is exceedingly complicated, and opens a field for extensive investigations. A very instructive résumé of the subject has been made by Dale (1924).

STATUS OF THE PROTOZOA IN THE ANIMAL KINGDOM.

It is usual to regard the Protozoa as constituting a Phylum which corresponds in status to one of the various Phyla, such as the Mollusca, Arthropoda, Vertebrata, etc., into which the rest of the animal kingdom is divided. This is the view adopted by most zoologists, but Dobell and O'Connor (1921) have recently expressed the view that the Protozoa constitute a group of organisms which has a status equal to the rest of the animal kingdom. According to Dobell's contention, discussed earlier in this work, the Protozoa are non-cellular animals, while the rest of the animal kingdom includes all cellular animals. On this account he divides the animal kingdom into two sub-kingdoms—the Protozoa and the Metozoa. Such a distinction may still be admitted, though there would be less reason for its recognition if the generally accepted view were held that the Protozoa are unicellular, and not merely non-cellular animals. Dobell, having raised the Protozoa to the rank of sub-kingdom, raises to the status of Phyla the various classes in which they are divided. For purposes of this work, however, it is unnecessary to discuss this very intricate subject, and, following the more orthodox view, the Protozoa will be still regarded as constituting a Phylum.

PART II

SYSTEMATIC DESCRIPTION OF THE PROTOZOA

WITH SPECIAL REFERENCE TO PARASITIC AND COPROZOIC FORMS

CLASSIFICATION OF THE PROTOZOA.

PHYLUM: **PROTOZOA**

SUB PHYLUM:
PLASMODROMA

CLASS: **RHIZOPODA**

Order: AMŒBIDA
„ HELIOZOA
„ RADIOLARIA
„ FORAMINIFERA
„ MYCETOZOA

CLASS: **MASTIGOPHORA**

SUB-CLASS: **Phytomastigina**

Order: CHRYSOMONADIDA
„ CRYPTOMONADIDA
„ DINOFLAGELLATA
„ EUGLENOIDIDA
„ PHYTOMONADIDA

SUB-CLASS: **Zoomastigina**

Monozoic Forms

Order: PROTOMONADIDA
„ HYPERMASTIGIDA
„ CYSTOFLAGELLATA

Diplozoic Forms

Order: DIPLOMONADIDA

Polyzoic Forms

Order: POLYMONADIDA

CLASS: **CNIDOSPORIDIA**

Order: MYXOSPORIDIIDA

Sub-Order: **Eurysporea**
„ **Sphærosporea**
„ **Platysporea**

Order: MICROSPORIDIIDA

Sub-Order: **Monocnidea**
„ **Dicnidea**

Order: ACTINOMYXIDIIDA

UNDETERMINED

SARCOSPORIDIA
GLOBIDIUM
HAPLOSPORIDIA

CLASS: **SPOROZOA**

SUB-CLASS: **Coccidiomorpha**

Order: COCCIDIIDA

Sub Order: **Eimeriidea**
„ **Hæmosporidiidea**
„ **Piroplasmidea**

Order: ADELEIDA

Sub-Order: **Adeleidea**
„ **Hæmogregarinidea**

SUB-CLASS: **Gregarinina**

Order: SCHIZOGREGARINIDA
„ EUGREGARINIDA

Sub Order: **Acephalinidea**
„ **Cephalinidea**

SUB-PHYLUM: **CILIOPHORA**

GROUP 1: PROTOCILIATA

CLASS: **OPALINATA**

GROUP 2: EUCILIATA

CLASS: **CILIATA**

SUB-CLASS: **Aspirigera**

Order: HOLOTRICHIDA

Sub-Order: **Astomatea**
„ **Stomatea**

Section 1: GYMNOSTOMATA
Section 2: TRICHOSTOMATA

SUB-CLASS: **Spirigera**

Order: HETEROTRICHIDA
„ OLIGOTRICHIDA
„ HYPOTRICHIDA
„ PERITRICHIDA

CLASS: **SUCTORIA**

PHYLUM: PROTOZOA GOLDFUSS, 1817.

THE phylum Protozoa, as defined above, is the subdivision of the animal kingdom in which all unicellular animals are grouped. It may be divided into two sub-phyla, as suggested by Doflein (1901). The first of these is the **PLASMODROMA,** which includes the forms which have pseudopodia or flagella, and in which syngamy, where it is known to occur, consists in the complete fusion of two gametes. The second sub-phylum is the **CILIOPHORA,** which comprises those Protozoa which have numerous cilia as motile organs, a special type of binuclearity (macronucleus and micronucleus), and a process of syngamy in which two individuals temporarily associate, undergo exchange of nuclei, and then separate. The class Opalinata, in which syngamy is of the type seen amongst the Plasmodroma while the binuclearity characteristic of the other classes of the Ciliophora is wanting, forms a connecting link between the two sub-phyla.

A. *SUB-PHYLUM:* PLASMODROMA DOFLEIN, 1901.

This, the first of the sub-phyla into which Doflein divides the Protozoa, includes forms which have either pseudopodia or flagella as organs of locomotion, and the parasitic Sporozoa which, owing to their mode of life, have been modified in various ways. There is either a single vesicular nucleus or more than one are present. Syngamy takes place by the complete fusion of gametes, which may be alike (isogamy) or different (anisogamy). In many forms, after a period of asexual reproduction, syngamy, followed by a different method of reproduction, occurs (alternation of generations).

The sub-phylum contains four classes of Protozoa, two of which include mainly free-living forms, while two contain forms which are exclusively parasitic. One class is characterized by the amœboid form of the body which produces pseudopodia as organs of locomotion, while in another, though the body may be amœboid, it possesses one or more flagella. The Protozoa of the first type belong to the class **RHIZOPODA,** and those of the second to the class **MASTIGOPHORA.** The separation of these two classes is rendered difficult by the fact that certain organisms which are amœboid and devoid of flagella for the greater part of their existence may at certain stages develop flagella, while, conversely, forms which usually possess flagella may have a purely amœboid phase.

As regards the parasitic types, many observers have grouped them together in the one class Sporozoa, which was divided by Schaudinn

(1900) into the Telosporidia and Neosporidia. It appears, however, that these two groups are so fundamentally different that it is better to follow Hartmann (1907) and place the Neosporidia in a separate class, for which Doflein's name Cnidosporidia may be employed, and to reserve the Sporozoa for the forms included in Schaudinn's group Telosporidia. The class **CNIDOSPORIDIA** includes parasitic Protozoa, which are either amœboid or almost, if not entirely, motionless. They produce, by a complicated process of development in which several cells take part, very characteristic encysted stages or spores which are peculiar in possessing special bodies called polar capsules, from which long filaments can be extruded. The class **SPOROZOA** also comprises parasitic forms, which reproduce characteristically by schizogony. After syngamy the zygote gives rise to a number of sickle-shaped sporozoites. These are either free within the oöcyst which forms around the zygote, or they are enclosed in a number of secondary cysts, the sporocysts, which are formed inside the oöcyst. Schaudinn included the Sarcosporidia in his group Neosporidia. These parasites, however, have little in common with the true Cnidosporidia, and though they produce bodies which are called spores, these are structurally quite different from those of the Cnidosporidia. In fact, very little is known about the true nature of the Sarcosporidia, which will be considered with certain other parasitic forms (Haplosporidia, Globidium) of undetermined affinities.

B. *SUB-PHYLUM:* CILIOPHORA DOFLEIN, 1901.

Ciliophora is the name given by Doflein to the second of the two subdivisions into which he divides the Protozoa. The organisms included in this group have a comparatively complex structure, and in this respect may be considered to be the most highly specialized of the Protozoa (Fig. 70). The body is not, as a rule, subject to changes of shape, unless as a result of external pressure, there being a definite body form for each individual. The most characteristic feature is the possession of numerous hair-like processes, the cilia, which cover either the whole or only part of the body surface. The cilia are used as organs of locomotion, or for producing currents in the water for the intake of food. They may also serve as organs of special sense, such as taste or touch.

The cytoplasm is differentiated into an endoplasm, which contains the nuclei, contractile vacuoles, and food vacuoles, and a highly-organized ectoplasm. The latter consists of a superficial membrane, the pellicle, within which is a layer containing myonemes or contractile fibres, spaces and canals of an excretory system, basal granules from which the cilia arise, and sometimes *trichocysts*, which are small bodies from which

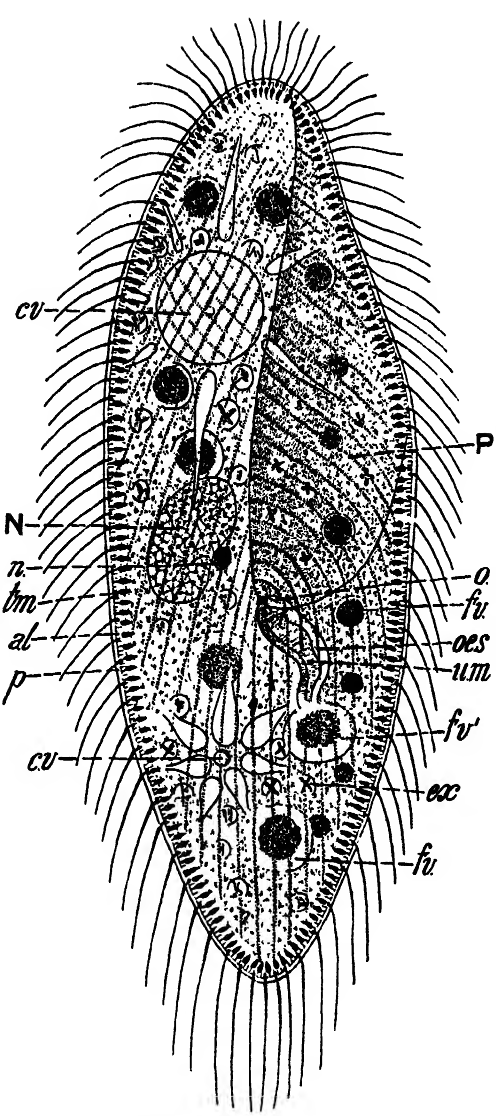


FIG. 70.—DIAGRAMMATIC FIGURE OF *Paramecium caudatum* (× *ca.* 500). (FROM MINCHIN, 1912, AFTER LANG.)

P., Peristome groove: *o*, mouth; *œs.*, œsophagus with undulating membrane; *f.v.'*, food vacuole forming at end of œsophagus; *f.v.*, other food vacuoles; *c.v.*, contractile vacuole with surrounding channels leading to it; *ex.*, excretory crystals; N, macronucleus; *n*, micronucleus; *tm*, trichocysts; *al.*, alveolar layer; *p.*, pellicle; *um*, undulating membrane

threads are discharged. A definite mouth opening or cytostome may, or may not, be present.

Though the Ciliophora agree with one another in the possession of cilia, they differ fundamentally as regards their nuclei. In what may be regarded as the more primitive forms (Opalinata) there are present in each individual two or more nuclei which are all of one type, in which respect an approach to the Plasmodroma is made. When syngamy occurs uninucleated forms are produced, and these, which are gametes, unite in pairs, with complete fusion of the bodies and nuclei. In other forms there are typically two morphologically distinct nuclei, one of which is a macronucleus and the other a micronucleus. During syngamy the macronucleus disintegrates and takes no part in the process, while the micronucleus divides. Two individuals associate, and one of the daughter micronuclei of each individual migrates into the other and unites with its remaining daughter micronucleus. When this has taken place, the associated or conjugating individuals separate and continue to lead an independent existence. On the basis of this distinction Metcalf (1918) recognizes two groups, the PROTOCILIATA and the EUCILIATA. The members

of the group Protociliata (**OPALINATA**) possess cilia during the whole of their existence, whereas amongst the Euciliata certain forms (**CILIATA**) constantly have cilia, while others (**SUCTORIA**) have them only in their youngest free-swimming stages, which, however, soon attach themselves to objects, lose their cilia, and develop suctorial tentacles.

Multiplication amongst the Ciliophora is by binary fission or bud formation. Amongst the multinucleated Protociliata nuclear division proceeds somewhat irregularly, and division of the body leads to the production of two daughter multinucleated individuals, which may, or may not, possess an equal number of nuclei. In the case of the Euciliata, which typically possess one macronucleus and one micronucleus, both these nuclei divide, so that each daughter individual possesses a pair of nuclei similar to that of the parent.

From the foregoing remarks it will be seen that the phylum Protozoa may be subdivided as follows:

A. *SUB-PHYLUM:* **PLASMODROMA** DOFLEIN, 1901.—Movement is effected by pseudopodia or flagella, and syngamy, where it is known, takes place by the complete fusion of gametes.

I. *CLASS:* **RHIZOPODA** von Siebold, 1845.—The predominating phase is amœboid, locomotion being effected by means of pseudopodia.

II. *CLASS:* **MASTIGOPHORA** Diesing, 1865.—The predominating phase is flagellate, locomotion being effected by means of flagella.

III. *CLASS:* **CNIDOSPORIDIA** Doflein, 1901.—Parasitic forms which are frequently amœboid, but which produce characteristic spores provided with polar capsules from which long filaments can be extruded.

IV. *CLASS:* **SPOROZOA** Leuckart, 1879.—Parasitic forms which reproduce typically by schizogony, and which give rise to sporozoites enclosed in resistant oöcysts after syngamy has occurred.

B. *SUB-PHYLUM:* **CILIOPHORA** DOFLEIN, 1901.—Movement is effected by means of cilia.

GROUP 1: PROTOCILIATA Metcalf, 1918.—There are two or more nuclei, which are all of one type. Syngamy is effected by the complete fusion of uninucleated gametes.

I. *CLASS:* **OPALINATA.**—With the characters of the group.

GROUP 2: EUCILIATA Metcalf, 1918.—There is a definite nuclear dimorphism, the nuclei being of two types (macronuclei and micronuclei). When syngamy takes place the macronuclei disintegrate, the micronuclei alone taking part in the process, which is characterized by the exchange of the products of division of the micronuclei between two temporarily associated individuals.

I. *CLASS:* **CILIATA** PERTY, 1852.—Cilia are present throughout the life of the organism.

II. *CLASS:* **SUCTORIA** CLAPARÈDE AND LACHMANN, 1858.—Cilia are present only during the young stages, which usually attach themselves to objects, lose their cilia, and develop suctorial tentacles.

A. *SUB-PHYLUM:* PLASMODROMA.

I. *CLASS:* RHIZOPODA v. SIEBOLD, 1845.

CLASSIFICATION.

CLASS: **RHIZOPODA**
- *Order:* AMŒBIDA
  - *Family:* AMŒBIDÆ.
    - *Genus:* **Amœba.**
    - „ **Hartmannella.**
    - „ **Vahlkampfia.**
    - „ **Sappinia.**
    - „ **Pelomyxa.**
    - „ **Entamœba.**
    - „ **Endamœba.**
    - „ **Endolimax.**
    - „ **Iodamœba.**
    - „ **Dientamœba.**
  - *Family:* PARAMŒBIDÆ.
    - *Genus:* **Paramœba.**
  - *Family:* DIMASTIGAMŒBIDÆ.
    - *Genus:* **Dimastigamœba.**
  - *Family:* RHIZOMASTIGIDÆ.
    - *Genus:* **Mastigamœba.**
    - „ **Mastigella.**
    - „ **Mastigina.**
- *Order:* HELIOZOA
- „ RADIOLARIA
- „ FORAMINIFERA
  - *Genus:* **Chlamydophrys.**
- *Order:* MYCETOZOA

The Protozoa belonging to the class Rhizopoda (= Sarcodina Hertwig and Lesser, 1874) are typically organisms which move, and ingest food by means of pseudopodia. These are cytoplasmic processes of varying form which are protruded from the surface of the body, and which, after fulfilling their function, are withdrawn. They may be merely short, stumpy elevations, or more elongate finger-like processes (Fig. 5). Sometimes they are very fine, and give the organism a radial appearance. Such radiating pseudopodia, seen typically amongst the Heliozoa, may be supported by stiff axial fibres, which cause them to be more permanent structures (Fig. 71). There may be but a single pseudopodium, another one being protruded only when the first has been withdrawn; several may be developed at one time, or large numbers are produced simultaneously from the whole surface of the body. In the latter case, anastomoses may be formed between adjacent pseudopodia, so that the organism has the appearance of being surrounded by a loose network of cytoplasm. They may be shorter than the diameter of the body, or many times this length. The cytoplasm may be differentiated into a

superficial clear hyaline layer, the ectoplasm, and a more granular fluid, endoplasm. A pseudopodium may be formed of ectoplasm alone, or it may have a core of endoplasm. Within the endoplasm are to be found the nuclei, food vacuoles, and various granules, while contractile vacuoles are present in the forms which are not parasitic.

In some Rhizopoda (Foraminifera) the ectoplasm secretes a protective shell known as a theca, which covers the body almost entirely (Fig. 72). A pore is left, through which pseudopodia are protruded, to enable the organism to move about and secure its food. In addition to the main opening, the shell may be perforated by numerous minute pores. Shells of this kind may be formed when the organism is only partially grown, and with increase in size a new and larger shell is made. With further growth others still larger are produced, and these, remaining attached to one another, give rise to many chambered shells, the separate sections of which are variously arranged according to the particular species. The Radiolaria have a perforated membranous central capsule, which divides the cytoplasm into a central mass in which the nucleus lies, and an extracapsular portion or mantle. In the latter siliceous skeletal structures of various kinds are developed. These take the form of shells or spicules, which are often conspicuous for the beauty of their design. Whatever may be the character of the organism, the predominating phase in development is one which produces pseudopodia, and in the majority no other phase is known to exist. In some, however, a transitory flagellate phase occurs, during which the organism resembles in every respect a member of the class Mastigophora. On this account it is exceedingly difficult to define accurately the limits between the two classes Rhizopoda and Mastigophora. In the latter the flagellate phase is the predominating one, while in the former it is the pseudopodial or amœboid phase. It has been demonstrated in the case of certain organisms (*Dimastigamœba*) that the amœboid or flagellate phase can be pro-

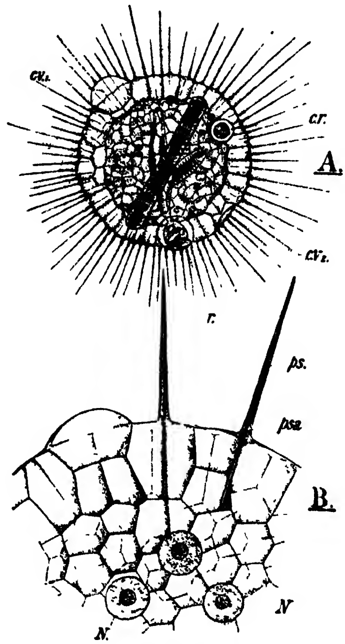


FIG. 71.—*Actinosphærium eichhorni:* AN ENTIRE INDIVIDUAL (× 90) AND PORTION OF ANOTHER (× 360). (FROM LANKESTER'S *Treatise on Zoology*, AFTER LEIDY, 1879.)

c.v.$_1$, Contractile vacuole; *c.v.*$_2$, position of another contractile vacuole which has just collapsed; *cr.* food vacuole; *r.*, rotifer just engulfed; *ps.*, pseudopodium; *psa.*, axis of pseudopodium; N., nucleus.

duced by altering the character of the medium in which the organisms are growing.

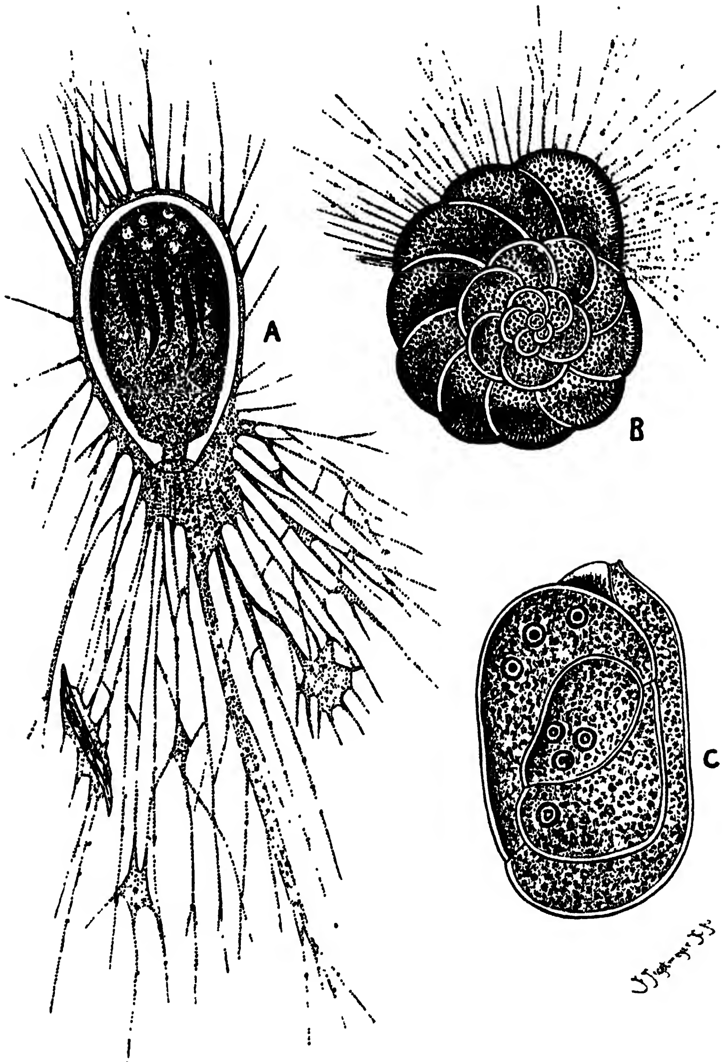


FIG. 72.—TYPES OF SHELLED RHIZOPODA. (FROM LANG, 1901, A AND B, AFTER MAX SCHULTZE; C, AFTER R. HERTWIG.

A. *Gromia oviformis* with ingested *Navicula* and seven nuclei (× *ca.* 50). The pseudopodia round the shell should be three times as long as represented.
B. *Rotalia freyeri*, showing spirally arranged chambers (× *ca.* 90).
C. *Spiroloculina* sp., showing four chambers and nuclei (× *ca.* 30).

In some instances (*Mastigamœba, Mastigina, Mastigella*) the body of the organism resembles an amœba, in that pseudopodia are formed for

the purpose of locomotion and ingestion of food, while a flagellum is present as a permanent structure (Fig. 73). Such organisms, though usually placed amongst the Rhizopoda, might with equal justification be classed with the Mastigophora.

The majority of the Rhizopoda possess a single nucleus, which divides only when multiplication occurs. In some cases, however, two nuclei are present, while in others the organisms are multinucleate. Some of the multinucleate forms (Mycetozoa) are relatively large, each consisting of a sheet of cytoplasm (plasmodium) easily visible to the naked eye. Re-

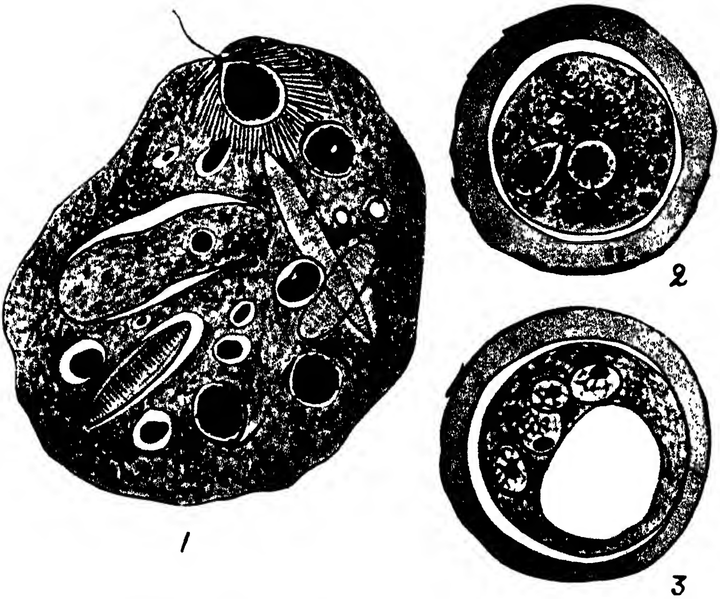


FIG. 73.—*Mastigina hylæ:* FREE AND ENCYSTED STAGES (× 1,275). (AFTER COLLIN, 1913.)

1. Free amœboid form with four nuclei, to one of which a flagellum is attached.
2. Encysted form with two nuclei.
3. Encysted form with four nuclei.

production amongst Rhizopoda usually takes place by binary fission, or simple division into two more or less equal parts. In association with encystment, when a protective capsule is formed around the organism, the single nucleus, by repeated divisions, may give rise to a number of nuclei, and the multinucleate cytoplasmic body within the cyst then segments into a corresponding number of daughter individuals. The latter may be amœboid organisms, like the adults from which they were derived, or they may be flagellated bodies which swim about for some time before losing their flagella and again becoming amœbæ. In the case of some of the Foraminifera, there is a complicated life-cycle involving

an alternation of generations (Fig. 74). Thus, in *Polystomella crispa*, a many-chambered shelled form studied by Lister (1895) and Schaudinn

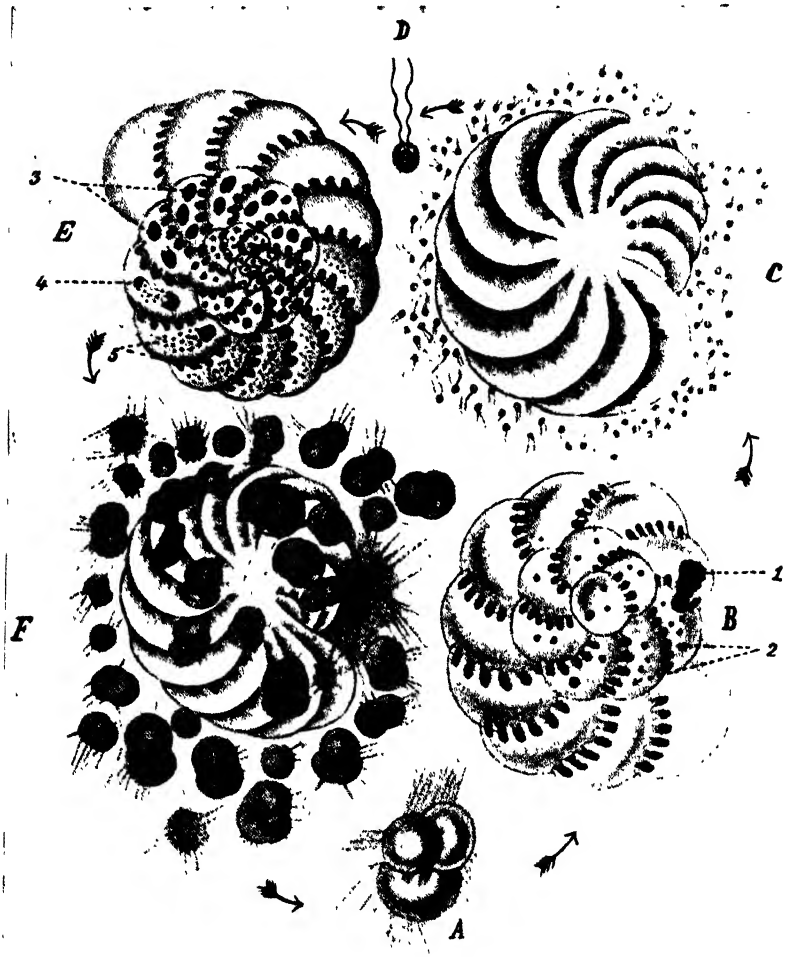


FIG. 74.—STAGES IN THE LIFE-CYCLE OF *Polystomella crispa* (× *ca.* 70). (AFTER LANG, 1901.)

A. Young megalospheric individual with three chambers.
B. Fully-grown megalospheric individual.
C. Megalospheric individual in process of formation of flagellated spores.
D. Flagellated spore more highly magnified. E. Fully-grown microspheric individual.
F. Microspheric individual in process of formation of daughter amœboid forms which become megalospheric forms.
1-3, nuclei of various sizes; 4, fragmenting nucleus; 5, chromatin granules.

(1895), the individual (microspheric form) becomes multinucleate, and then gives rise to a number of daughter amœboid forms which escape from the shell (Fig. 74, E and F). Each of these forms a relatively large

shell, and grows into a many-chambered individual of another type (megalospheric form), while the cytoplasm within the shell again gives rise, by multiple segmentation, to daughter individuals (Fig. 74, A to C). In this case, each daughter form which escapes from the shell is provided with two flagella, by means of which it swims about till it meets another similar form which has been produced by another individual. Conjugation takes place, and the zygote, losing the flagella, becomes an amœba, which forms a small shell and grows into a many-chambered individual of the first type (microspheric form). In the great majority of the Rhizopoda, however, no sexual process has been observed.

The class Rhizopoda may be sub-divided into the five orders:

AMŒBIDA, HELIOZOA, RADIOLARIA, FORAMINIFERA, AND MYCETOZOA.

1. *Order:* AMŒBIDA CALKINS, 1902.

The body consists of cytoplasm unprotected by any shell or skeletal structure, while movement is effected by the formation of pseudopodia from any part of the body surface. There is usually a single nucleus, but some forms have two and others many nuclei. The cytoplasm is generally differentiated into a softer and vacuolated inner portion, the endoplasm, in which the nucleus and food materials lie, and an outer, more hyaline, and clearer layer, the ectoplasm. This order includes the organisms which are generally known as amœbæ, and to it belong the various parasitic forms which occur in the intestinal canal of man and animals.

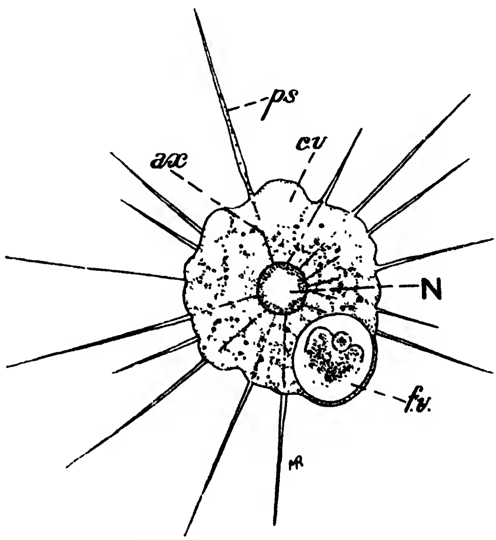


FIG. 75.—*Actinophrys sol* (× *ca.* 600). (FROM MINCHIN, 1912, AFTER GRENACHER.)

N., Nucleus from which radiate the axial fibres of the pseudopodia; *ps.*, pseudopodia; *ax.*, axial fibres; *c.v.*, contractile vacuole; *f.v.*, food vacuole.

2. *Order:* HELIOZOA HAECKEL, 1866.

The forms included in this order have a characteristic radial appearance, the result of fine spiky pseudopodia (axopodia), which are stiffened and rendered permanent by axial fibres. The latter may radiate from a granule, probably centrosomic in nature, situated at the centre of the organism, while the nucleus lies to one side of this (Fig. 51). The

Heliozoa are popularly known as sun animalcules, and are mostly found in fresh water. Two common forms are *Actinosphærium eichhorni* (Fig. 71), which is multinucleated, and *Actinophrys sol* (Fig. 75), which has a single nucleus. Both have been much studied from the point of their nuclear divisions and pedogamy, as described above (p. 86). Members of the genus

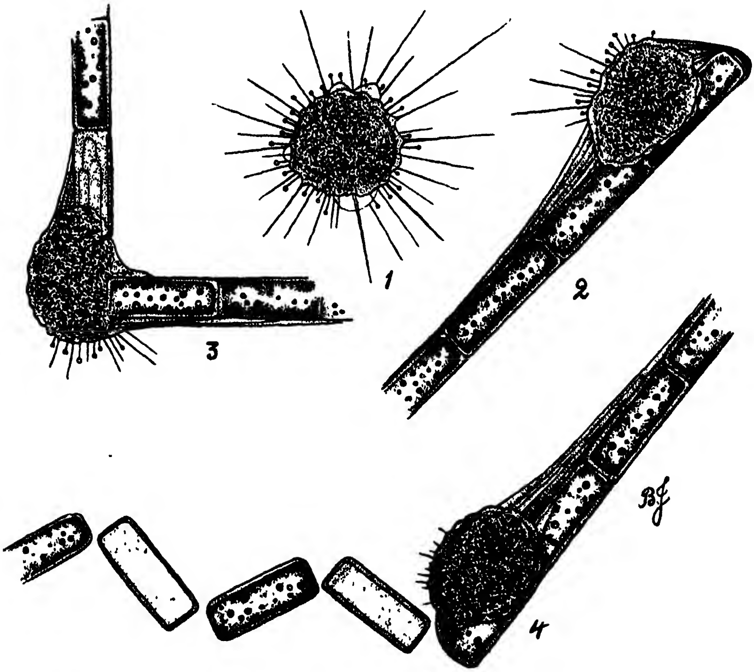


FIG. 76.—*Vampyrella lateritia:* A SINGLE INDIVIDUAL AT DIFFERENT STAGES OF ITS ATTACK ON AN ALGA (×300). (AFTER CASH, 1905.)

1. The free individual. 2. The same applied to the surface of the filament.
3. The filament has been broken, and one segment evacuated.
4. Later stage with four segments detached, two of which are evacuated.

Vampyrella are parasitic forms which bore their way into the cells of algæ, in which they live and multiply (Fig. 76). Another genus, *Nuclearia*, parasitizes not only algæ, but also other Protozoa.

3. *Order:* RADIOLARIA HAECKEL, 1861.

The members of this order, like those of the preceding one, show a tendency towards a radial arrangement of the pseudopodia, but morphologically they are more complicated than the Heliozoa. Various skeletal structures are commonly produced, while a perforated membranous structure, the capsule, divides the cytoplasm into a central intracapsular portion, which contains the nucleus, and an extra-

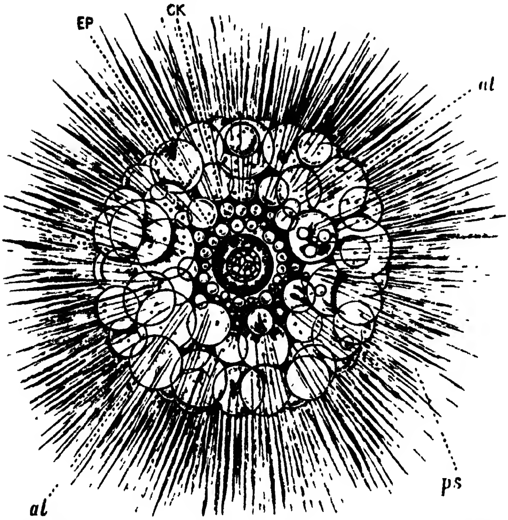


FIG. 77.—*Thalassicola pelagica:* AN INHABITANT OF THE OCEAN SURFACE WATERS (× 25). (FROM GAMBLE'S ARTICLE IN LANKESTER'S *Treatise on Zoology*, 1909.)

CK, Central capsule; EP, extracapsular cytoplasm; *al*, carbonic acid filled vacuoles (alveoli); *ps*., pseudopodia.

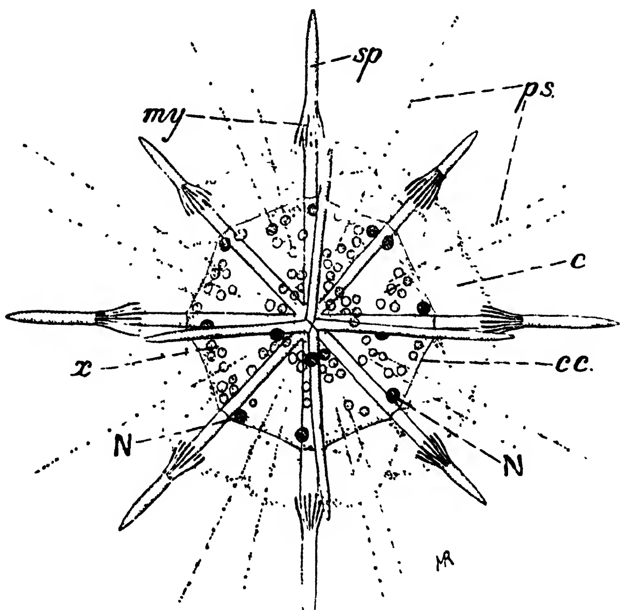


FIG. 78.—*Acanthometra elastica* (× *ca.* 150). (FROM MINCHIN'S *Protozoa*, 1912.)

sp. Radiating spines; *ps*., pseudopodia; *c*., calymma; *c.c*., central capsule; N., nuclei; *x*, yellow cells; *my*., myophrisks (rod-like bodies).

capsular portion (Fig. 77). The skeleton may be in the form of radiating spines, tangentially arranged rods, or definite fenestrated shells (Fig. 78). The latter may be spherical, with perforations, and several such shells may be formed concentrically, one within the other, as the animal increases in size, or they may have a definite axis, and be shaped like a cone or bottle. In many forms the cytoplasm contains peculiar yellow cells about 15 microns in diameter. These are known as zooxanthellæ, and each is an independent vegetable organism possessing a cellulose wall and containing a nucleus and chloroplasts. It is probable that they live in a condition of symbiosis with the host. The Radiolaria are marine organisms which are found floating on the surface of the ocean. Their shells are found in large numbers in the deposits of the ocean bed.

4. *Order:* FORAMINIFERA D'ORBIGNY, 1826.

These Rhizopoda (= Testacea Schultze, 1854) may be regarded as amœbæ which have the body protected by an external shell or theca. In the simplest forms the shell has a single opening, through which the organism protrudes pseudopodia for locomotion purposes and the capture of food, very much

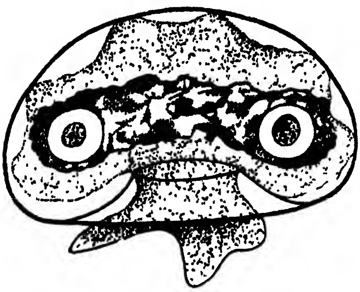


FIG. 79.—*Arcella vulgaris*, SHOWING OUTLINE OF SHELL, SIDE VIEW OF THE CIRCULAR CHROMIDIAL BODY, AND TWO NUCLEI ($\times$ 1,000). (ORIGINAL.)

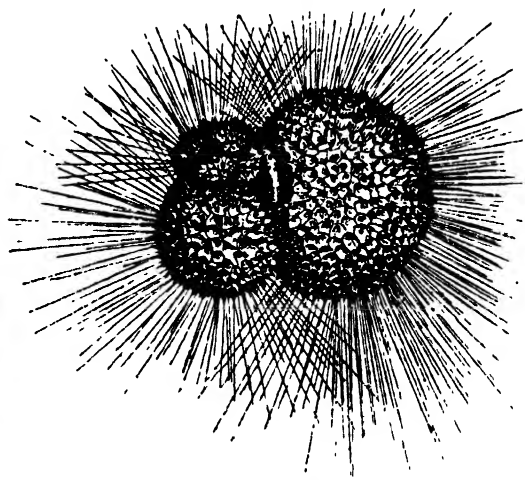


FIG. 80.—*Globigerina bulloides* FROM OCEAN SURFACE WATERS ($\times$ 70). THE SHELLS FORM THE MAIN CONSTITUENT OF THE "GLOBIGERINA OOZE" OF THE OCEAN BED. (AFTER RHUMBLER, FROM LISTER'S ARTICLE IN LANKESTER'S *Treatise on Zoology*, 1903.)

as a snail emerges from its shell (Imperforata Carpenter, 1862). Such forms (*Arcella, Difflugia*, etc.) are very commonly found in stagnant water (Fig. 79). The shells may be strengthened by adherent sand grains or other material (Fig. 8). When reproduction is to take place the nucleus divides, a portion of cytoplasm with one of the daughter nuclei is protruded through the opening, a new shell is formed around this, and another shelled individual

separated by division of the cytoplasm. In other cases, with growth of the organism, a new and larger shell, which remains adherent to the original one, is formed to accommodate it. A succession of new shells may be produced, and these remain attached to one another in such a way as to give rise to complicated compound shells which are constant in arrangement for any particular species. In addition to the main aperture the shells may have numerous minute pores, through which filose pseudopodia may be protruded (Perforata Carpenter, 1862) (Fig. 80). Reproduction of the simpler forms is by binary fission, while the more complicated types may show an alternation of generations with the production of flagellated gametes, as described above (p. 164). The Foraminifera occur either in fresh water or in the sea. The simpler ones occur in the former situation, while the more complicated types are marine forms. Chalk deposits are composed largely of shells of marine Foraminifera (Fig. 81). Those which occur in fresh water are often placed in a separate order, Thecamœbida (Delage and Hérouard, 1896), but there is no sharp line of demarcation between these and the true marine Foraminifera. Some forms, such as *Chlamydophrys*, may pass through the intestine of an animal in the encysted condition, and emerge from the cyst and develop their characteristic thecæ in fæces after they have left the body.

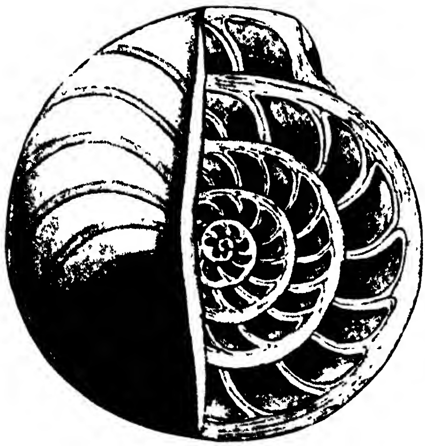


FIG. 81.—SHELL OF *Nummulites cummingii* (× 20). PORTION OF WALL REMOVED TO SHOW THE CHAMBERS. (FROM LANG, 1901, AFTER BRADY.)

Chlamydophrys stercorea Cienkowsky, 1875.—This shelled amœba is of interest, as it is commonly present in fæces of such animals as horses and pigs, as well as frogs and toads. In the freshly passed fæces, it occurs in the encysted condition which has passed through the intestine. If the fæces are kept moist for a few days or planted on agar plates, the amœbæ emerge from their cysts and secrete a thin, egg-shaped, transparent shell, which has a pore at its narrower end, through which the organism protrudes pseudopodia (Fig. 82). There is a single nucleus with a large central karyosome. Dobell (1909) gave the measurements of an average-sized individual as 20 by 14 microns. The writer, who has obtained cultures from frogs' fæces as well as from dirty water, has observed forms which are much smaller than this, some of them being barely 15 microns in length. The organisms readily encyst. If they

have no shell, they merely become spherical and form a cyst; if they are shelled, they escape from the cyst first. The cysts vary from 6 to 17 microns in diameter. Multiplication takes place by division of the nucleus, followed by the extrusion, through the pore, of half the cytoplasm into which one of the nuclei passes. A new shell is secreted round this portion with its pore directed towards that of the original shell. Finally, division of the narrow neck of cytoplasm uniting the two shelled individuals takes place.

Schaudinn (1903) stated that the cysts of *Chlamydophrys stercorea* passed through the human intestine, and that sometimes the amœbæ escaped from their cysts and multiplied while still in the intestine. He

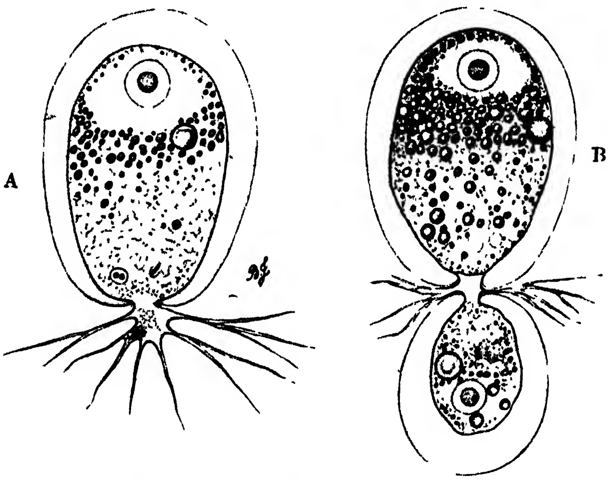


FIG. 82.—*Chlamydophrys stercorea* FROM PIGS' FÆCES (× 1,000). (ORIGINAL.)

A. Ordinary individual. Clear area round nucleus is the chromidial body.
B. Process of binary fission: daughter individual being formed as a bud.

also made the statement that a supposed amœba, *Leydenia gemmipara*, which Leyden and Schaudinn (1896) had found in human ascitic fluid, was no other than the free amœboid stage of *Chlamydophrys stercorea* which had wandered from the intestine to the peritoneal cavity. There seems to be no evidence of this whatever, and as Schaudinn was unaware of the existence of such parasitic forms as *Endolimax nana*, it is highly probable that the amœbæ he saw in the human intestine and regarded as *C. stercorea* were in reality *E. nana*. As to the nature of *Leydenia gemmipara*, there is no reason to suppose that it was anything more than body cells in a degenerate condition in the peritoneal exudate.

Bělař (1921) has reviewed the genius *Chlamydophrys*, and concludes there are six distinct species, which differ from one another in size, method

of nuclear division, and other details. *C. stercorea*, according to him, measures from 30 to 40 microns in length.

Nöller, Krosz, and Arndt (1921) have cultivated from horse and pig dung a number of thecamœbæ belonging to the genera *Chlamydophrys*,

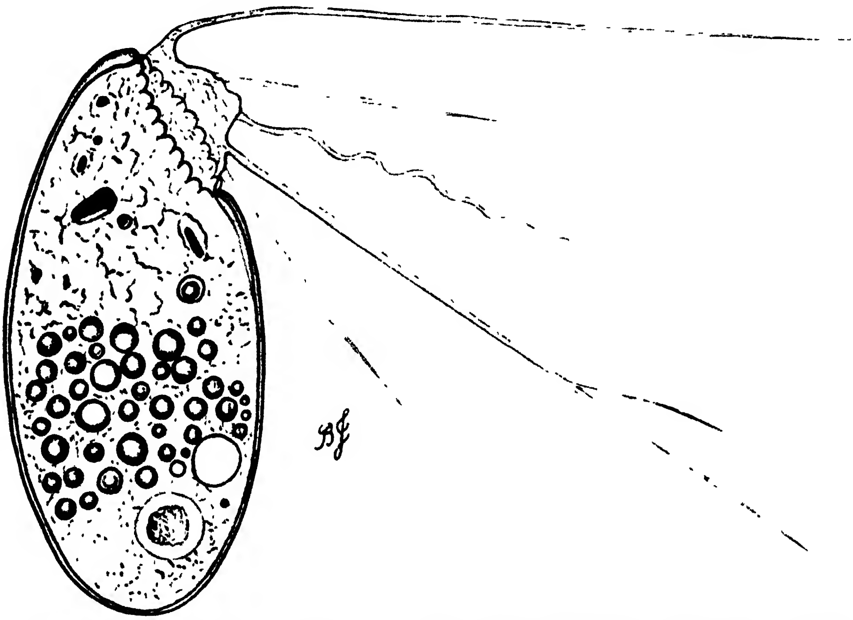


FIG. 83.—*Trinema acinus*: A SHELLED RHIZOPOD FROM POND WATER (×2,000). (ORIGINAL.)

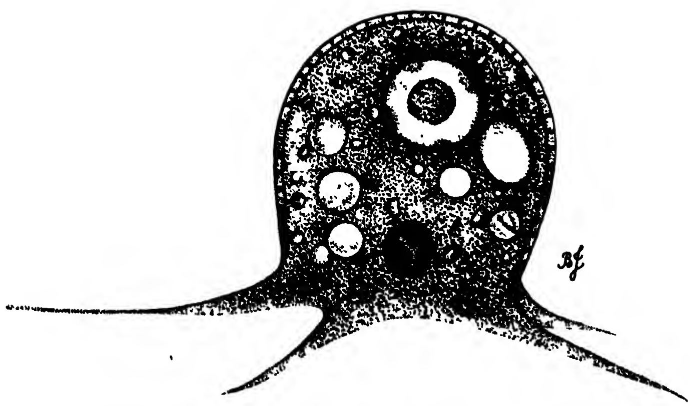


FIG. 84.—*Cochliopodium bilimbosum* (×1,000). (AFTER LEIDY, 1879.)

Plagiophrys, *Trinema* (Fig. 83), *Gromia*, and *Cochliopodium* (Fig. 84). Many of these forms multiply readily on agar plates. If pigs' fæces are

kept moist in a Petri dish for some days, many of these forms appear along with other coprozoic Protozoa.

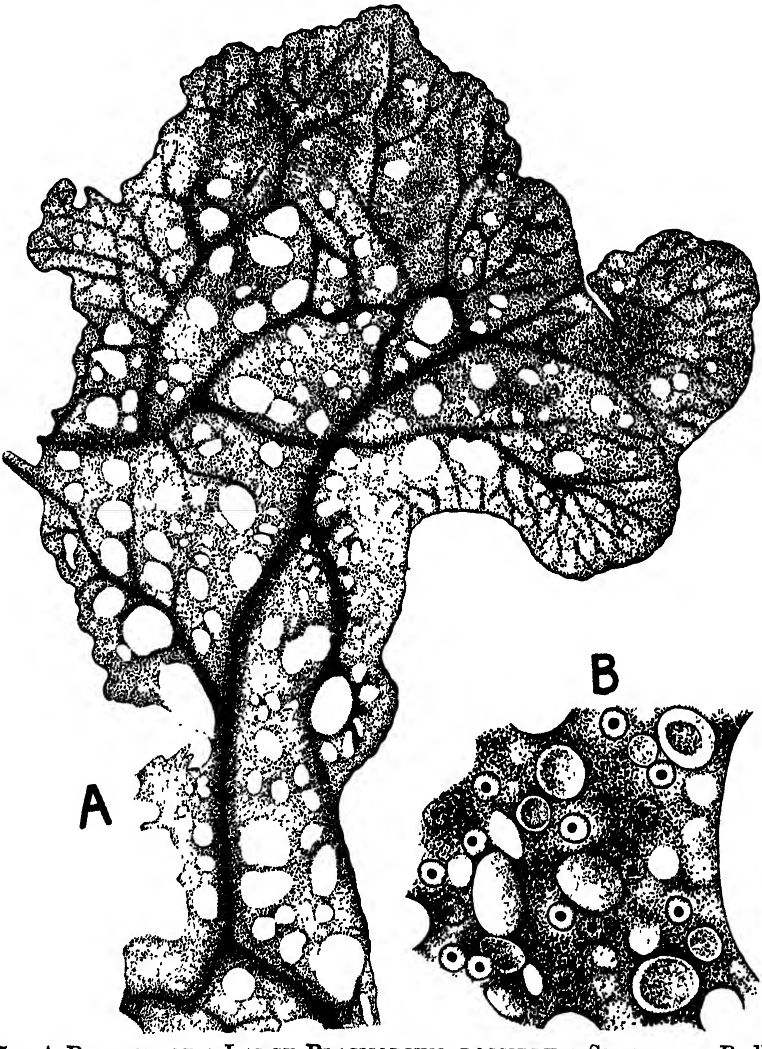


FIG. 85.—A PORTION OF A LARGE PLASMODIUM, POSSIBLY A SPECIES OF *Badhamia*, WHICH WAS GROWN ON AN AGAR PLATE. (ORIGINAL.)

A. General appearance under low magnification (× 16).
B. Small portion more highly magnified, showing numerous nuclei and vacuoles with inclusions (× 1,000).

5. *Order:* MYCETOZOA DE BARY, 1859.

The forms included in this order are characterized by a plasmodial adult phase. The plasmodium is a large sheet of multinucleated cytoplasm which exhibits peculiar streaming movements associated with the

production of branching and anastomosing pseudopodia (Fig. 85). At certain stages, portions of the cytoplasm become encysted in resistant capsules (sporangia), which may be arranged on stalks (Fig. 86). In this respect there is a striking resemblance to fungi, to which group the Mycetozoa were originally thought to belong. The sporangia eventually rupture, and may liberate flagellated organisms which, after a free-living existence, assume the amœboid form. By growth, accompanied by nuclear multiplication, the large plasmodia are produced. The Mycetozoa are terrestrial in habit, and are commonly found on the moist surfaces of decaying wood and leaves, or in similar situations. Some of them may be grown on the surface of agar plates.

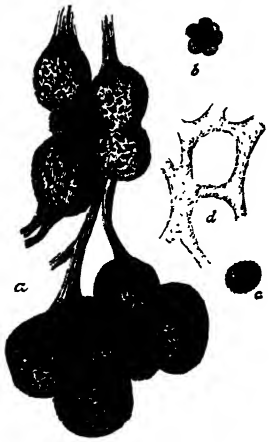


FIG. 86.—*Badhamia utricularis.* (AFTER LISTER, IN LANKESTER'S *Treatise on Zoology*, 1909.)

a. Group of sporangia (× 12).
b. Cluster of spores (× 170).
c. Single spore.
d. Part of capillitium in interior of sporangium (× 170).

SYSTEMATIC DESCRIPTION OF THE ORDER AMŒBIDA.

From the point of view of parasitology it is chiefly members of the order Amœbida which have to be considered. The vast majority of the Rhizopoda are free-living organisms, and only a comparatively small number are truly parasitic and adapted to their hosts in such a way that a free extra-corporeal existence does not occur. The fact that many of the free-living non-parasitic forms are able to produce protective cysts of a resistant nature to enable them to withstand desiccation has led to some confusion. Such encysted forms are frequently eaten accidentally by human beings or animals, and may pass unharmed through the intestinal canal. After escape from the body in the dejecta, they may find themselves in an environment which is favourable for further development. The amœbæ emerge from the cysts, and by active multiplication increase enormously in numbers in a comparatively short time. In this way, erroneous impressions as to their parasitic nature may be obtained. Care must always be exercised to guard against the possibility of confusing these coprozoic forms with true parasites. In the case of true parasites, the only forms which survive outside the body are, as a rule, the encysted forms, which remain quite passive and unchanged till they are ingested by another host. The unencysted stages are present in the freshly passed stool, and show a degeneration which becomes more marked as the interval since their escape from the body increases. The non-

parasitic forms, which have passed through the alimentary canal in the encysted state, are at the height of their free-living existence some time after the escape of the cysts from the body.

In the order Amœbida are included a number of well-known free-living amœbæ, such as *Amœba proteus* (Fig. 5) and *Amœba verrucosa* (Fig. 87). The majority are uninucleated, but some have two nuclei (*A. binucleata*), while others have many nuclei (*Pelomyxa*). In addition to these larger forms, there are others which are smaller, and which are of interest in that some of them are readily cultivated from the fæces of man and animals, owing to the fact that their cysts are able to pass unharmed through the intestine. Such forms are known as coprozoic amœbæ. They have frequently been referred to as *Amœba limax*, a name given to an amœba by Dujardin (1841) which, according to Dobell and O'Connor (1921), is not now identifiable. Some of the amœbæ ascribed to this species have been shown to be the amœboid phase of the flagellated organism *Dimastigamœba gruberi* mentioned below. Others appear to be true amœbæ which have no flagellate stage. There are many species which are difficult to identify on account of their resemblance to one another. They differ in the character of the cysts they produce, the method of nuclear division, and other details.

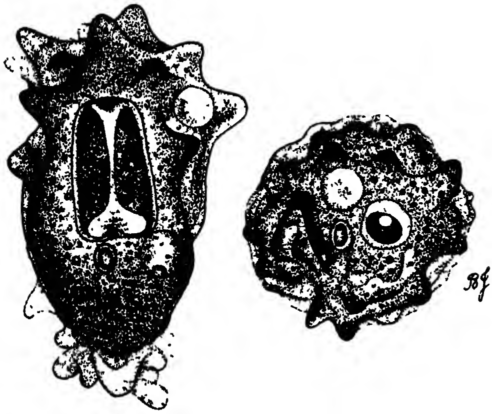


FIG. 87.—*Amœba verrucosa* (× 300). (AFTER CASH, 1905.)

The order Amœbida may be considered as comprising the following families:

1. *Family:* AMŒBIDÆ Bronn, 1859.—Amœbæ which are not able to form flagella.

2. *Family:* PARAMŒBIDÆ Poche, 1913.—Amœbæ which, in addition to a nucleus of the usual type, possess an accessory body (Nebenkörper) which, during division, divides with the nucleus.

3. *Family:* DIMASTIGAMŒBIDÆ.—Amœbæ which in the adult form are able under certain conditions to form two or more flagella, by means of which they progress as flagellates.

4. *Family:* RHIZOMASTIGIDÆ Calkins, 1902.—Amœbæ which are provided with a single flagellum during the greater part of the free-living existence.

Family: AMŒBIDÆ Bronn, 1859.

In this family are included a number of free-living amœbæ, and most of the parasitic forms which occur in the intestine of man and animals. Not many years ago all amœbæ, including the parasitic forms, were placed in the genus *Amœba*. It is now recognized that several distinct genera are represented, but the group has not been sufficiently studied to enable precise definitions to be given. Many of the smaller free-living forms which were grouped under the name *Amœba limax* have been placed in the genera *Hartmannella, Sappinia, Vahlkampfia*, which can be identified by the type of nuclear division and other details, while the parasitic amœbæ have been separated into the genera *Entamœba, Endamœba, Iodamœba, Endolimax*, and *Dientamœba*. The exact limits of the genus *Amœba* are doubtful, but the majority of the large free-living uninucleated forms, such as *Amœba proteus* and *Amœba verrucosa*, which may have a diameter of 500 microns or more, are regarded as belonging to it. Much more information regarding the complete life-histories, the methods of reproduction and encystment, and the details of nuclear division, are required before the group can be satisfactorily defined.

Genus: **Amœba** Bory, 1822.

In this genus are included the vast majority of free-living amœbæ. In most cases they are placed in the genus because detailed information regarding their structure and development is wanting. It seems probable that future investigators will show that the only ones which actually belong to it are the large free-living forms like *Amœba proteus, Amœba verrucosa, Amœba vespertilionis*, and *Amœba hydroxena* described by Entz (1912) as parasitic on *Hydra oligactis*.

Genus: **Hartmannella** Alexeieff, 1912.

The amœbæ belonging to this genus are recognized by the character of their nuclei and method of nuclear division. The nucleus is spherical, has a large central karyosome, and peripheral chromatin in the form of granules either on the inner surface of the nuclear membrane or in the space between the membrane and karyosome. During division the karyosome disintegrates, and a spindle is formed upon which definite chromosomes become arranged as an equatorial plate (Fig. 88). The nuclear membrane usually disappears at some stage of the division. The cysts are spherical structures. The numerous species belonging to this genus are distinguished by the details of nuclear division and the character of the cysts.

Hartmannella hyalina (Dangeard, 1900).—This amœba, which is often found in stale fæces or in agar plate cultures made from dirty water,

fæces, or other material, has been referred to by various observers as *Amœba hyalina*, a name given to it by Dangeard (1900). The generic name *Hartmannella* was created by Alexeieff (1912*a*). The amœba which was cultivated from human fæces by Musgrave and Clegg (1904) in the Philippines, the one described by Liston and Martin (1911) in

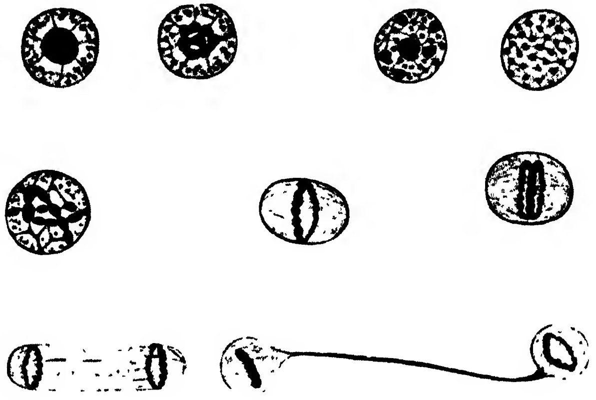


FIG. 88.—STAGES IN THE NUCLEAR DIVISION OF A SPECIES OF *Hartmannella* ISOLATED FROM PIGS' FÆCES ($\times$ *ca.* 3,400). (ORIGINAL.)

India as occurring in culture media inoculated with liver abscess pus, and water, and the form growing on plates after exposure to the air, as noted by Wells (1911), are probably this species.

The amœba, when spherical, has a diameter of 9 to 17 microns. It has a contractile vacuole, while the nucleus consists of a nuclear membrane

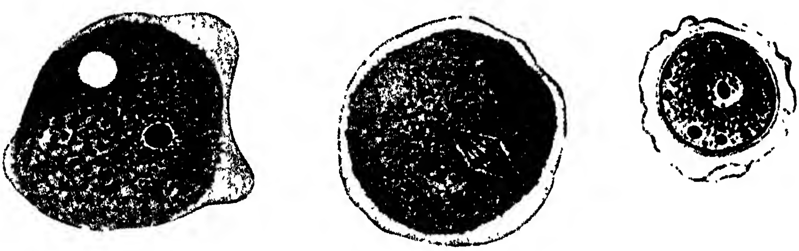


FIG. 89.—*Hartmannella hyalina* ($\times$ 2,000). (AFTER DOBELL AND O'CONNOR, 1921.)

1. Ordinary amœba.
2. Division stage, showing pointed spindle with equatorial plate of chromosomes.
3. Cyst with crinkled wall.

and large central karyosome (Fig. 89). Peripheral chromatin granules occur on the nuclear membrane, and in the clear zone between it and the karyosome. At the time of division the karyosome disintegrates, and a spindle is formed, at the equator of which the chromatin, in the form

of a ring of spherical chromosomes, is arranged. The nuclear membrane disappears during the process, leaving a sharp, pointed spindle in the cytoplasm. The spherical cysts measure from 10 to 14 microns in diameter. They have a smooth inner wall and a much wrinkled outer one. The amœba does not multiply within the cyst, nor does its nucleus undergo division. When grown on the surface of agar, it not infrequently happens that amœbæ with two or more nuclei encyst, in which case a corresponding number of nuclei occur within the cyst.

There are other amœbæ of relatively small size belonging to the genus *Hartmannella*, which differ from one another in the details of their nuclear divisions. Thus *H. glebæ*, described by Dobell (1914 *a*), is very similar to *H. hyalina* (Fig. 56). The spindle formed during nuclear division has, however, rounded ends instead of pointed ones. The cyst, moreover, has a smooth outer surface. This form, or one closely allied to it, often occurs coprozoically in fæces, and can be cultivated on agar plates (Fig. 56). At the present time it is impossible to identify many of these coprozoic amœbæ, but it appears that two fairly well-defined types commonly occur—the one corresponding to *H. hyalina*, and the other to *H. glebæ*.

Genus: **Vahlkampfia** Chatton and Lalung-Bonnaire, 1912.

Vahlkampf (1905) studied the development of an amœba, which he designated *Amœba limax*. The nucleus possessed a large central karyosome, and during multiplication the nucleus divided with the formation of pole caps, as in the case of *Dimastigamœba gruberi* (Figs. 61 and 90). A similar form was named *Amœba punctata* by Dangeard (1910). Chatton and Lalung-Bonnaire (1912) created the genus *Vahlkampfia* for amœbæ showing this type of nuclear division and possessing pores in the cyst wall. Flagellate forms of the amœbæ were not observed, but the conditions necessary for the production of the flagellate forms were not provided by them. Several other observers have described amœbæ which show nuclear division of the same type, and which have not been noted to give rise to flagellate forms. It does not seem improbable that most, if not all, of these forms would produce flagellate stages if the necessary conditions existed. *Dimastigamœba gruberi* remains as an amœba on agar plates, or in cultures in egg-albumen water and other media, and does not become a flagellate unless a sudden change occurs in the medium, as, for instance, that produced by the addition of tap water. If this is done, flagellates appear in three or four hours, but they revert to the amœboid form again in about a day (Fig. 120). It is probable that many of the amœbæ which have been placed in the genus *Vahlkampfia* would be capable of transformation into flagellate forms if they were similarly treated. It is possible, however, that some of them would not. Hogue (1921), for example, obtained a

culture of an amœba, which she named *Vahlkampfia patuxent*, from the stomach of oysters. Though its method of nuclear division resembled that of *Dimastigamœba gruberi*, she failed entirely to obtain flagellate forms, though all the methods which cause *Dimastigamœba gruberi* to develop flagella were tried.

Calkins (1913) separated the amœbæ which have this particular type of nuclear division into two genera—viz., the genus *Vahlkampfia*, to include the forms which do not develop a flagellate stage, and the genus *Nægleria* (created by Alexeieff, 1912) for those which have such a stage. The latter forms, as pointed out by Alexeieff (1912*a*) really belong to the genus *Dimastigamœba* of Blochmann (1894), and will be considered

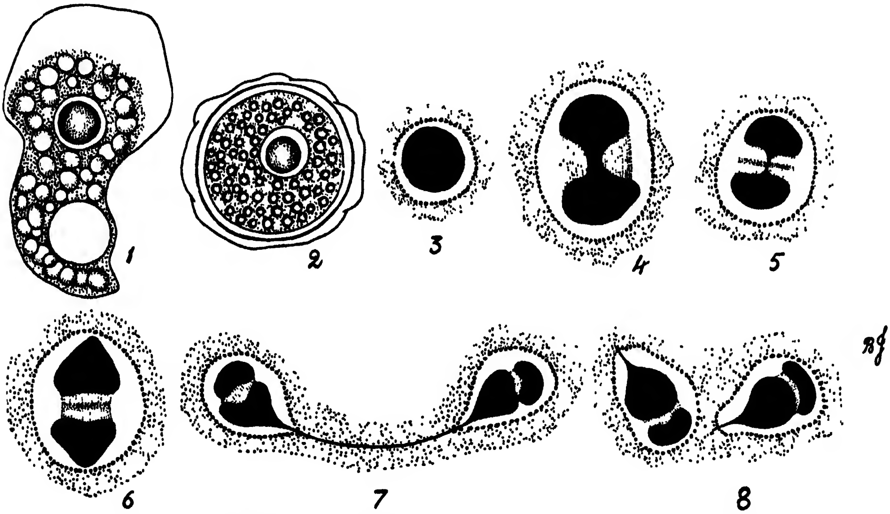


FIG. 90.—*Vahlkampfia punctata*. (AFTER VAHLKAMPF, 1905.)
1, 2. Appearance of living amœba and encysted form (× 1,500 ?). 3-8. Stages in nuclear division (× 3,000 ?).

below (p. 260), and as Chatton and Lalung-Bonnaire actually observed markings which were undoubtedly pores on the cyst wall, it is probable they were dealing with an organism belonging to the same genus. In this case, both the names *Nægleria* and *Vahlkampfia* are really synonyms of *Dimastigamœba*.

As already remarked, it is still doubtful if any of the amœbæ having the type of nuclear division of *Dimastigamœba gruberi* are really incapable of developing the flagellate stage. The majority, at any rate, have not been investigated from this point of view. Most of these forms are free-living amœbæ, occurring commonly in damp soil or decomposing vegetable material, but some of them have been found in the intestines of cold-blooded animals. Others are to be regarded as coprozoic amœbæ, as they

appear in stale fæces, and have been cultivated from stools on agar plates. A large number have been named, but it is very doubtful if these are all distinct species.

Dangeard (1910) described as *Amœba punctata* a form of this type which had cysts with punctate markings. It was studied by Chatton and Lalung-Bonnaire (1912), who obtained it from human fæces. They placed it in a new genus as *Vahlkampfia punctata*. The punctate markings strongly suggest the pores in the cysts of *Dimastigamœba gruberi*.

Hartmann (1907*a*) gave the name *Amœba froschi* to an amœba showing the same type of nuclear division which he had seen in the fæces of frogs, and the name *Amœba lacertæ* to a similar form in the intestinal contents of lizards of the genus *Lacerta*. Both these forms were studied by Nägler (1909). The form described by Dobell (1914*a*) as *Amœba lacertæ*, which also occurred in the intestinal contents of lizards, differed as regards the details of its nuclear division from the form studied by Hartmann and Nägler. Hartmann (1914) accordingly renamed the form studied by Dobell *Amœba* (*Vahlkampfia*) *dobelli*. Caullery (1906) gave the name *Amœba pædophthora* to an amœba which parasitized the eggs of the marine crustacean *Peltogaster curvatus*, while Chatton (1909) described, under the name *Amœba mucicola*, an amœba which was parasitic on the gills of a marine fish. Epstein and Ilovaisky (1914) gave the name *Vahlkampfia ranarum* to a large amœba, reaching 50 microns in diameter, which they found in the intestine of frogs. Mackinnon (1914) saw an amœba, which she referred to as *Vahlkampfia* sp., in the intestine of the larvæ of the crane-fly, *Tipula* sp. An amœba, which was cultivated by Whitmore (1911*a*) from human fæces, liver-abscess pus, and tap water in Manila, and referred to as *Amœba limax*, was placed in the genus *Vahlkampfia* as *V. whitmorei* by Hartmann and Schilling (1917). The amœba described by Porter (1909*a*) as *Amœba chironomi*, from chironomous larvæ, is possibly of the same type, though the nuclear division was not described. Hogue (1921) recorded *V. patuxent* from the stomach of oysters in America.

In addition to the above-mentioned forms, which have a certain association with higher animals, a number of free-living species have been named. Nägler (1909) described *Amœba spinifera*, *A. lacustris*, and *A. albida*; Aragão (1909), *A. diplomitotica*; Gläser (1912), *A. tachypodia*; Bělař (1915), *A. diplogena*; Jollos (1917), *Vahlkampfia magna*, *V. debelis*, and *V.* sp.; and Hogue (1914), *Vahlkampfia calkensi*. Gläser (1912) described the nuclear division of Ehrenberg's *Amœba verrucosa* as being of the *Vahlkampfia* type. An amœba first seen by Molisch (1903), and later by Zacharias (1909), is parasitic on *Volvox*, while another, *Amœba blochmanni* (Doflein, 1901), first noted by Blochmann (1886), is parasitic on

Hæmatococcus. It is possible that both these forms, as well as the others named above, should be included in the genus *Vahlkampfia.*

These various amœbæ all agree with one another in that the nuclear division, where it has been studied, is of the type first described by Vahlkampf (1905), and it is highly probable that further investigations will demonstrate, in some of them at least, the presence of pores in the cyst wall and the occurrence of flagellate stages, in which case they will have to be transferred to the genus *Dimastigamœba.* Meanwhile, however, till more accurate data are forthcoming, it seems advisable to group these amœbæ under the name *Vahlkampfia,* which, as pointed out above, may be a synonym of *Dimastigamœba,* rather than to establish a new genus, which will be necessary if they are finally proved to have no flagellate stage.

Genus: Sappinia Dangeard, 1896.

The amœbæ belonging to this genus are peculiar in possessing two nuclei, which are closely applied to one another. During division, both

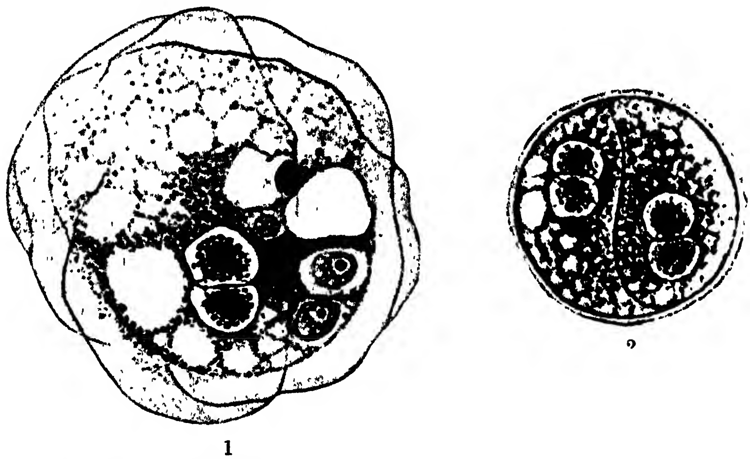


FIG. 91.—*Sappinia diploidea* (×2,000). (AFTER DOBELL AND O'CONNOR, 1921.)

1. Ordinary individual with two nuclei in apposition.
2. Cyst containing two individuals.

nuclei divide. When encystment takes place, two amœbæ, each with two nuclei, are enclosed in a common cyst. In the form *S. pedata,* studied by Dangeard (1896 *a*), the free amœbæ have the characteristic two nuclei. The cyst, however, is peculiar in having a pedicle or stalk attaching it to objects.

Sappinia diploidea (Hartmann and Nägler, 1908).—This is an amœba which was isolated by Hartmann and Nägler from lizards' fæces. According to Dobell and O'Connor (1921), it occurs rarely in human fæces, but more

commonly in that of animals, such as the ox and lizard. Hartmann and Nägler (1908) gave it the name *Amœba diploidea*, while Alexeieff (1912*a*) placed it in Dangeard's genus *Sappinia*. The amœba varies in size from 10 to 30 microns, possesses a contractile vacuole, and has a characteristic thick pellicle, which is sometimes wrinkled (Fig. 91). It possesses two nuclei which lie side by side in a central position. They are spherical, and have large central karyosomes. The amœba multiplies by binary fission, the two nuclei dividing and producing two parallel spindles. The daughter individuals thus have two nuclei. When the amœbæ encyst, two individuals form round themselves a common cyst. According to Hartmann and Nägler, the two nuclei of each amœba now fuse. Each nucleus is then said to give off reduction bodies, which degenerate, after which the cytoplasms of the two uninucleate amœbæ unite. Their nuclei, however, come into contact with one another, but do not fuse (Fig. 47). The amœba emerges from its cyst, and commences to multiply by binary fission as before (see p. 82).

AMŒBÆ OF PLANTS.

Franchini (1922 *g*, *h*, *j*, *k*, *l*) in a series of papers stated that he had found amœbæ in the latex of various plants. They occurred either alone or in association with flagellates of the leptomonas or trypanosome type. The plants found infected were Euphorbias, figs, and allied forms, as well as the lettuce, and were as follows: *Euphorbia verticillata, Euphorbia nereifolia, Chlorocodon Whitei, Cryptostegia grandiflora, Strophanthus Rigali* and *S. scandens, Acokanthera venenata, Thevetia* sp., *Cerbera Odollam, Ficus Benjamina, Ficus Pierrei, Ficus Tholloni, Ficus carica, Ficus parietalis, Antiaris toxicaria, Lakoocha artocarpus, Chrisophyllon* sp., *Labramia Bojeri, Tregulia Africana, Mimusops schimperi, Sideroxylon inerme, Lactuca sativa, Plumeria alba.*

Cultures of some of the amœbæ were obtained by inoculation of blood-agar plates (Nöller's medium) with the latex of the plants, and in this medium most of the amœbæ were found to ingest red blood-corpuscles. In this way cultures were made from *Ficus carica, Chlorocodon Whitei, Cryptostegia grandiflora, Acokanthera venenata, Plumeria alba*, and the lettuce, *Lactuca sativa*. Three of these amœbæ were named *Amœba chlorocodonis, Amœba cryptostegiæ*, and *Amœba lactucæ*. The descriptions of the amœbæ and the figures are such that it is impossible to form an opinion as to their nature. It seems not improbable that the cultures obtained may have been derived from amœbæ or their cysts on the cuticle of the plants.

Further remarkable assertions are made by the author (Franchini, 1922 *n*) in connection with the inoculation of kittens with cultures of amœbæ from the plants *Acokanthera venenata* and *Plumeria alba*. Kittens injected *per rectum* with cultures of these amœbæ were said to remain well for six to ten days, when they suddenly became ill with dysentery, which persisted for about ten days. During this period amœbæ, some of which included red blood-corpuscles, were constantly present. The animals recovered. The figures of these amœbæ, again, are unrecognizable, and cannot be distinguished from cells. It is further claimed that mice are susceptible to inoculation with the amœbæ cultivated from latex of Euphorbias, and that, when these cultures contain trypanosomes and leishmania, as well as the amœbæ, a general infection is produced, and that all these organisms can be recovered by culture from the heart blood. In another paper Franchini (1923) asserts that

mice fed on the latex of the plants or injected intraperitoneally with cultures of the amœbæ and flagellates acquired liver abscess in which amœbæ with included red corpuscles occurred. These amœbæ are said to reproduce by schizogony, and give rise to forms like anaplasma, leishmania, and trypanosomes. The figures purporting to show these forms are quite unrecognizable, and it is impossible to understand the author's conception of these Protozoa.

Genus: Pelomyxa Greef, 1874.

This genus includes certain large free-living multinucleate amœbæ, which may reach a diameter of 2 millimetres. They occur commonly in stagnant water, and are easily visible to the naked eye. Each individual, which may contain several hundred nuclei, moves slowly as it throws out blunt pseudopodia (Fig. 92). The character of the cytoplasm varies with the medium in which the organism is growing. It is usually much vacuolated, and may be packed with small globules of a refringent substance,

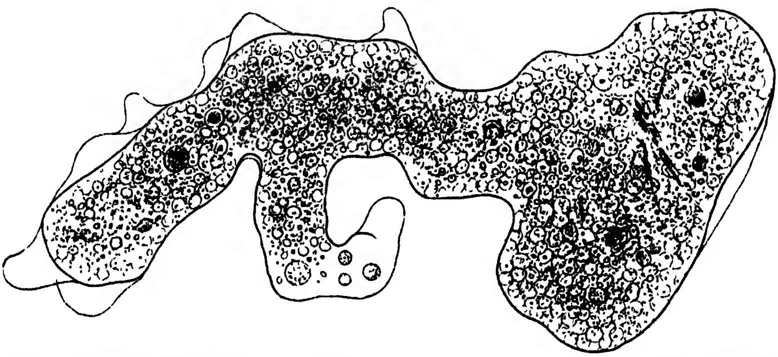


FIG. 92.—*Pelomyxa palustris:* ORDINARY CONDITION DURING ACTIVE MOVEMENT (× 1,000). (AFTER CASH, 1905.)

in addition to sand grains, diatoms, bacteria, and other objects, which cause it to be peculiarly opaque. At other times the globules are absent, and the cytoplasm is much clearer. Reproduction takes place by fission, while gamete formation has also been described by Bott (1906). It is supposed that uninucleated individuals are divided off, and that these conjugate in pairs to give rise to zygotes, which grow into the multinucleated adults (see p. 30).

Genus: Entamœba Casagrandi and Barbagallo, 1895.

The members of this genus, which inhabit the intestine of higher animals, vary in diameter from 5 to 40 microns. The pseudopodia are usually blunt processes, and a new one is rarely formed before the previously existing one is withdrawn. A central endoplasm can be distinguished from a peripheral ectoplasm, while a contractile vacuole is not present.

The nucleus is spherical, and consists of a definite nuclear membrane, on the inner surface of which the bulk of the chromatin of the nucleus is distributed in the form of granules. There is a linin network, upon which fine granules of chromatin may or may not occur, while a comparatively small karyosome is present. Reproduction in the vegetative phase is by simple binary fission, while transmission from host to host is effected by means of encysted forms. The cyst is a transparent and smooth structure, and the nucleus of the enclosed parasite, by repeated divisions, gives rise to a number of daughter nuclei, which vary from four to multiples of four. The encysted forms are passed out of the body of the host, and undergo no change till they enter the intestine of another host, where the cyst wall ruptures, and there is liberated either the multinucleated cytoplasmic body, which then divides into a number of amœbæ, or a number of amœbæ which have been formed before rupture of the cyst. It is not clear which of these processes actually occurs. Each species of the genus tends to produce a cyst which, when fully developed, contains a definite number of nuclei. Thus, *E. coli*, *E. mùris*, and other forms have eight, while *E. histolytica* and *E. ranarum* have only four. Occasionally, the nuclei are in excess of the usual number. There may be sixteen or more in *E. coli*, and more rarely eight in *E. histolytica.* The cysts of *E. ranarum* may have a still larger number. This tendency to nuclear excess may occur less commonly in the unencysted stages. In the case of *E. coli* unencysted forms with eight nuclei have been described, but it is probable that these were really irregularly-shaped encysted forms, the cyst walls of which, in stained preparations, were not actually visible. Multinucleate free forms of *E. ranarum* were described by Collin (1913), and similar stages were seen by Keilin (1917) in the case of *E. mesnili*, and by the writer in *E. histolytica.* It has been supposed by Mathis and Mercier (1917) that the cysts with an abnormally large number of nuclei represent a special type of multiplication by schizogony, while those with the normal number are destined to give rise to gametes. They produced no convincing evidence in support of this view. It is more probable that for some reason the nuclear multiplication has continued beyond the usual limits, possibly owing to excess of nutriment, or sometimes to an amœba having encysted just as it was about to divide in the free state.

This genus includes *Entamœba coli*, the harmless amœba of the human intestine; *E. histolytica*, the pathogenic form producing amœbic dysentery and liver abscess in man; *E. gingivalis*, an inhabitant of the human mouth; and various species which occur as intestinal parasites of animals, such as *E. muris* of rats and mice, *E. pitheci* and *E. nuttalli* of monkeys, *E. bovis* of cattle, *E. ovis* of sheep, *E. testudinis* of the tortoise, *E. ranarum* of frogs, *E. minchini* of the larvæ of Tipulid flies, and many other species. It is

probably safe to assume that practically every vertebrate animal, as well as many invertebrates, will be found to harbour amœbæ belonging to this genus. In the great majority of cases they are of the non-pathogenic variety, and in this respect resemble *E. coli*. In a few instances, forms associated with dysenteric symptoms have been described from animals. The various species resemble one another very closely, so much so that in many cases they could not possibly have been regarded as distinct species, apart from the fact that they occurred in different hosts.

There is some doubt as to the correct spelling of the name *Entamœba*. The generic title was created in this form by Casagrandi and Barbagallo (1895) for *E. coli* of the human intestine. Leidy (1879), however, had given the name *Endamœba blattæ* to the amœba of the cockroach. If this form should prove to belong to the same genus as the human amœba, then Leidy's name will have priority. As one of the cockroach amœbæ presents some peculiar features, it is better to regard it at present as belonging to a distinct genus, *Endamœba* (see p. 235).

ENTAMŒBÆ OF MAN.

(*a*) Pathogenic Form.

Entamœba histolytica Schaudinn, 1903.—Chief synonyms: "Amœba coli" Lösch, 1875; "Amœba dysenteriæ" Councilman and Lafleur, 1891; *Amœba coli* (Lösch) Kovács, 1892; *Amœba dysenteriæ* (Councilman and Lafleur) Kovács, 1892; *Entamœba dysenteriæ* (Councilman and Lafleur) Craig, 1905; *Entamœba coli* var. *tetragena* Viereck, 1907; *Entamœba africana* Hartmann, 1907; *Entamœba tetragena* (Viereck) Hartmann, 1908; *Poneramœba histolytica* Lühe 1909; *Entamœba minuta* Elmassian, 1909; *Entamœba nipponica* Koidzumi, 1909; *Entamœba hartmanni* Prowazek, 1912; *Löschia (Viereckia) tetragena* Chatton and Lalung-Bonnaire, 1912; *Entamœba brasiliensis* Aragão, 1912; *Löschia histolytica* (Schaudinn) Mathis, 1913; *Entamœba venaticum* Darling, 1915; *Entamœba minuta* Woodcock and Penfold, 1916; *Endamœba coli* (Lösch) Aragão, 1917; *Endamœba dysenteriæ* (Councilman and Lafleur) Pestana, 1917; *Entamœba tenuis* Kuenen and Swellengrebel, 1917; *Entamœba minutissima* Brug, 1917; *Endamœba histolytica* (Schaudinn) Craig, 1917; *Entamœba coli communis* Knowles and Cole, 1917; *Entamœba paradysenterica* Chaterjee, 1920; *Caudamœba sinensis* Faust, 1923; *Karyamœbina falcata* Kofoid (and Swezy, 1924); *Entamœba dispar* Brumpt, 1925.

Everyone who has studied the question is agreed that *E. histolytica* was first seen and described by Lösch (1875), and named by him "Amœba coli." Though this name was not correctly written in the original description given by Lösch, it was employed for a long time for the amœbæ of the human intestine before it was fully realized that more than one species existed. Similarly, Councilman and Lafleur (1891) proposed to call the amœba "Amœba dysenteriæ," another name which was not correctly presented. Quincke and Roos (1893) and Roos (1894) were the first to conclude that two types of amœba occurred in man, the one an active

form which produced cysts 10 to 12 microns in diameter and was pathogenic to cats, and the other a less active form which produced cysts 16 to 17 microns in diameter and which did not give rise to infection in cats. Kruse and Pasquale (1894) similarly described two forms, the one pathogenic to cats and the other not.

Though Schaudinn (1903), in his account of the amœbæ of the human intestine, made many erroneous statements, he was the first observer to appreciate clearly the fact that two distinct species exist, the one pathogenic and the other harmless. Before this, the descriptions referred sometimes to the one form and sometimes to the other, and often to a mixture of both. In many cases it is only the association of the amœbæ with pathological conditions, and their occurrence in lesions of the intestine and abscess of the liver, which are now known to be due only to invasion of tissues by *E. histolytica*, that make it almost certain that some of the earlier writers were actually dealing with this form. If recent investigations had shown that both *E. coli* and *E. histolytica* were liable to invade the tissues, then there would be practically no data whatever to enable a decision to be made as to which of the forms the earlier writers were referring. The experiences of the past few years have demonstrated clearly that *E. histolytica* alone is responsible for the production of pathological conditions, so that it is perfectly clear that the amœbæ described in the lesions of the intestine, liver, and brain by the earlier writers were actually *E. histolytica*, though the descriptions of the amœbæ themselves were in most cases so imperfect that it would be impossible to identify them. Though Lösch, in his original description, expressed a doubt as to the part played by the amœbæ in the production of dysentery, his really excellent figure depicts an organism which can hardly be any other than that now known as *Entamœba histolytica*.

If Schaudinn had recognized the fact that the amœba, which Lösch called "Amœba coli," was the pathogenic amœba, and had given it the name *Entamœba coli*, endless confusion would have been avoided, but as the matter stands at present there seems to be no alternative, unless further confusion is to be caused, but to retain Schaudinn's name *E. histolytica* for the pathogenic form and *E. coli* for the non-pathogenic one. The whole question of the nomenclature of the intestinal amœbæ of man has been reviewed very thoroughly by Dobell (1919), and readers are referred to his book for more detailed information on this very intricate subject.

LIFE-HISTORY.—*E. histolytica* is to be regarded as a tissue parasite of man, as first demonstrated by Koch and Gaffky (1887), and more clearly by Kartulis (1885 and 1886). Infection is brought about by the ingestion of encysted forms, first seen by Quincke and Roos (1893), which have been passed in the fæces of some other infected person. Under the action of

the digestive fluids the cyst ruptures. From the work of Chatton (1917*a*), who fed cats with material containing cysts, and that of Penfold, Woodcock, and Drew (1916), who treated cysts with *liquor pancreaticus,* it appears that it is the secretions in the small intestine which cause the cyst wall to dissolve.

Chatton stated that the cyst liberated a four-nucleated amœba, while the other observers merely noted that a single amœba escaped from the cyst. Whether this happens in the human intestine or not cannot be stated. Dobell and Stevenson (1918) and the writer have failed to bring about any escape of amœbæ from cysts by means of *liquor pancreaticus.*

From experiments on cats, there can be no doubt that human beings are infected by the ingestion of cysts. Whatever may be the exact method of escape of the encysted amœbæ and their development after this, it is a fact that invasion of the intestinal wall by the amœbæ quickly takes place. In the earliest condition the amœbæ make their way into the glands of the large intestine, and crawl to the bottom of these. Here they multiply, and partly by pressure, and possibly by the secretion of a toxin, the gland cells degenerate and separate from one another. By this time the tubule of the gland has probably become blocked, and if the adjacent glands over a small area of surface are all similarly involved, as is usually the case, a slightly raised yellowish nodule is produced. Meanwhile, the amœbæ have made their way into the interglandular connective tissue, and a certain amount of necrotic material from broken-down cells has collected. In this condition the yellow nodule is in reality a small amœbic abscess of the mucosa. Very soon this abscess bursts into the lumen of the intestine, and the contents are discharged, with the result that a small undermined ulcer is formed (Figs. 93, 94). The amœbæ which thus escape invade other glands, causing the condition to spread, or they are passed in the fæces with a certain amount of blood and mucus, which represents the discharge from the abscess. The infected portions of the intestine may be very limited, so that only a few scattered nodules are formed, or there may be a more or less continuous infection of all the glands. After rupture of the primary abscess the ulcer so formed becomes gradually larger, the amœbæ multiplying in the base of the ulcer and extending over a wider area. They break through the muscularis mucosæ, and extend into the submucous tissues, producing eventually ulcers which may reach an inch or more in diameter. These ulcers, like the small ones originally formed, have undermined edges, and become filled with mucoid material, débris of cells, and amœbæ. It is probable that the plugs of mucus admixed with blood, which occur in the stools of amœbic cases, represent the evacuations from these ulcers. These masses of mucus may contain enormous numbers of amœbæ.

At any time the discharged amœbæ may infect fresh areas, so that the large intestines of these cases show every stage in the formation of the ulcers, from the smallest yellow nodules to large, undermined ulcers.

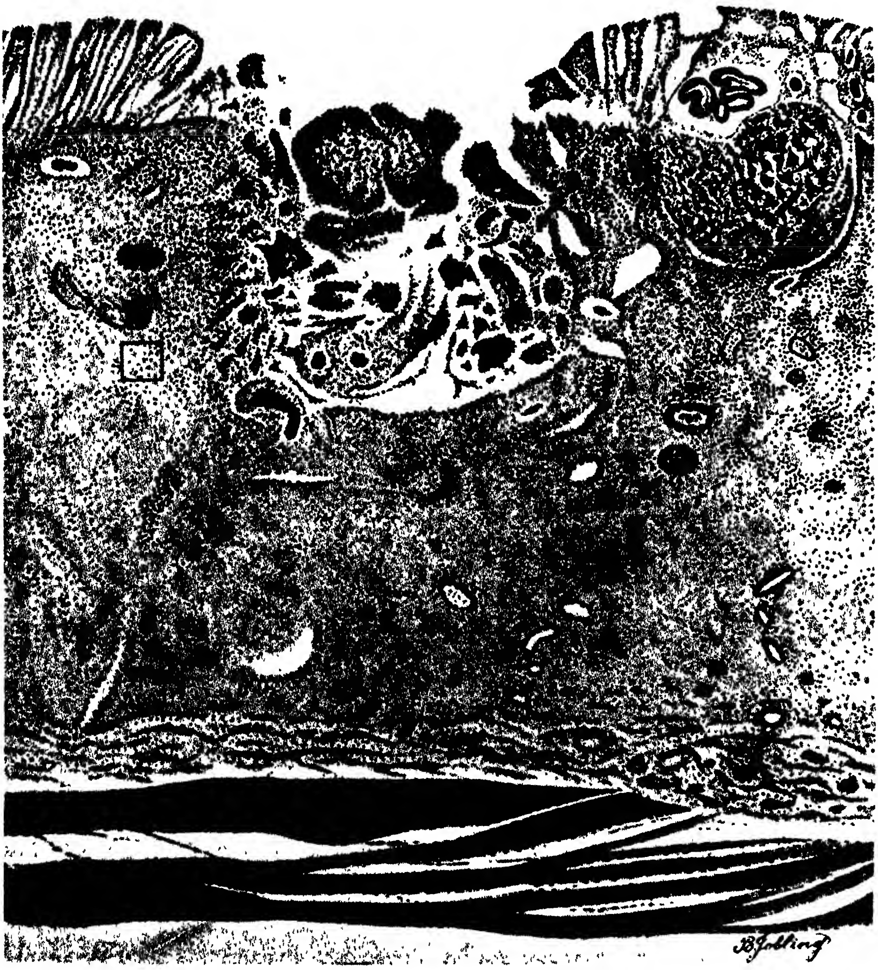


FIG. 93.—AMŒBIC ULCERATION: SECTION OF A SMALL ULCER IN A HUMAN LARGE INTESTINE (× 30). (ORIGINAL.)

The area marked by a square is shown under higher magnification at Fig. 94.

The best pictures from a histological point of view are obtained by cutting sections of the small nodules which have not yet become subject to secondary bacterial invasion, while at post-mortem examinations the contents of one of these small nodules will often show still live and active

amœbæ which cannot be obtained so readily from the larger ulcers. After the amœbæ have multiplied and caused an extension of the ulcer for some varying period a healing process sets in, the amœbæ disappear, and the site of the ulcer is finally represented by a puckered scar of fibrous tissue, while the peritoneal surface opposite it may be considerably thickened. In other situations, however, the process of invasion and ulceration is still continuing, and this affords an explanation of the persistence of infections with *E. histolytica*, which are known to last for

FIG. 94.—*Entamœba histolytica* IN TISSUE OF HUMAN LARGE INTESTINE (×500) (ORIGINAL.)

The area is shown in the square at Fig. 93.

many years, if not a lifetime, unless eradicated by suitable treatment. In their extension through the tissues of the intestinal wall the amœbæ not infrequently make their way into blood-vessels, and are carried as emboli to the liver, spleen, brain, or other organ, where they continue to multiply and give rise to the well-known amœbic abscesses.

In an infected individual, if the amœbæ are multiplying rapidly and invading one portion of the large intestine after another in quick succession, the discharge from the ulcers is considerable, and much blood and mucus will appear in the stool, which becomes of the characteristic

dysenteric type. If the extension is not rapid, then only occasional plugs of mucus, which may or may not be contaminated with blood, are passed, and the individual may be quite unaware of his condition. The cases of rapid extension are regarded as the acute ones, and the amœbæ are all of the large tissue-invading form, many of which contain red blood-corpuscles. In other cases, where there is not rapid extension, though a considerable area of the wall must be involved owing to the enormous number of amœbæ or their cysts which are passed in the fæces, a state known as the " carrier condition " occurs. Exactly what happens in this condition is not properly understood, for it is difficult to obtain perfectly fresh post-mortem material from these cases. It is not possible to reproduce the carrier condition in animals, which always acquire an acute infection which either terminates fatally or disappears. From what can be observed in the stool, it is found that a smaller type of amœba occurs in the fæces of carrier cases. These are in reality encysting forms (precystic amœbæ), for in association with them are to be found cysts showing one, two, or four nuclei. In some cases the precystic amœbæ and the encysted forms are passed together in the stool, while in others only the amœbæ or only the cysts are passed. This is probably dependent upon the varying rate at which the large intestine evacuates itself. It seems probable that the small amœbæ arise in the ulcers by division from the larger tissue-invading forms under certain conditions which may be supposed to hinder their free and easy development. As a general statement it can be accepted that Protozoa encyst when the conditions of life are becoming unfavourable. The small precystic amœbæ are formed from the large ones which have become more superficial in position, and it might be surmised that if the large amœbæ which have escaped from the tissues into the débris which fills the ulcer remain there for some time, as they may be supposed to do in the slowly extending cases, no increase in size occurs through lack of proper food, though they multiply and give rise at each division to increasingly small forms. These amœbæ, deprived of their proper food, which is to be found only in the tissues in the deeper parts of the ulcer, become encysted, and escape into the lumen of the intestine when the ulcer discharges its contents. This discharge may take place before actual encystment is complete, in which case the small precystic amœbæ will be found in the stool. In certain cases enormous numbers of cysts are passed in the stool, and it must be supposed that the process described occurs simultaneously at many parts of the intestine, not necessarily in large evident ulcers, but in the very small superficial ones which are not readily detected by the naked eye. The lesions in these cases may be merely superficial, and, not being of an acute nature, it is not surprising that certain individuals may be passing extraordinarily

large numbers of cysts without showing any symptoms whatever. In view of the recent successful culture of *E. histolytica* by Boeck and Drbohlav (1925) in egg media, it appears possible that the amœbæ may actually live and multiply on the surface of the intestine without giving rise to any lesions. It may be that the infection of many symptomless carrier cases is of this type, and that the precystic amœbæ and cysts are produced by amœbæ living on the surface of the mucosa.

As regards the fate of encysted amœbæ, there are two views. The one which maintains that an amœba which has once encysted in the gut is unable to leave its cyst in the large intestine of the same host appears to be in accord with the behaviour of parasitic Protozoa generally. According to this view an encysted amœba, in order to develop further, must pass out of the intestine and be ingested by another or the same host, so that the cyst may come under the influence of the digestive fluids of the small intestine. A corollary to this is that if all the amœbæ in an individual could be induced to encyst, an automatic cure would result, for all the cysts would have to be passed from the body. It is evident, therefore, that it is just as incorrect to suppose that any case is resisting treatment because of the impermeable cysts in the intestine as it would be to conclude that a case of ankylostomiasis was not cured because the eggs of the worm were too resistant. In the one case cure is effected by killing the amœbæ which produce the cysts, and in the other by killing the worms which produce the eggs. In either case the presence of cysts or eggs in the stool is an indication that the organisms producing them are still present in the intestine, and that treatment has so far failed to kill the organisms, and not that treatment has failed to kill the cysts or eggs. According to the second view, though the majority of cysts must necessarily escape from the intestine, some hatch in the large intestine before they escape, so that the encysted stage can be regarded as a resistant one. There is no evidence that this actually takes place in the large intestine of man, though Sellards and Theiler (1924) have succeeded in infecting kittens by injecting them *per rectum* with material which they claim contained only encysted forms of *E. histolytica*. Dr. Drbohlav informs the writer that he has been able to confirm this observation, which has been repeated by Hoare (1926). The writer has observed in stained preparations cysts of *E. histolytica* which appeared to have ruptured and to have developed hernia-like protrusions. It is just possible that this may be a natural process, and represents the escape of amœbæ from the cyst.

An individual who is in the carrier condition may at any time revert to one of acute amœbic dysentery. An infected person frequently suffers from periodic attacks of acute amœbic dysentery when only the large tissue-invading forms are present in the stool. Between the attacks,

when the acute symptoms have abated, the carrier condition maintains, when precystic amœbæ and cysts are passed. Certain individuals become infected without suffering from acute dysentery, the infection being detected only as a result of microscopic examination of the fæces. Those who become carriers after acute attacks have been termed *convalescent carriers* by Walker and Sellards (1913), and the others *contact carriers.* Such carriers may remain infected for many years, probably for the rest of their lives, without at any time being seriously troubled by their infection. In this respect the infections with *E. histolytica* are very similar to those produced by pathogenic bacteria.

As would be expected from the above account, the signs of an infection with *E. histolytica* vary considerably. In the acute condition, if there is extensive ulceration, the quantity of mucus and blood and the number of amœbæ passed in the stool may be considerable. The mucus is generally of a brownish colour and the blood of a dark red tint. If ordinary food has been continued, as is often the case, and the large intestine has not been emptied of fæcal matter, this will be present, and mixed with the blood and mucus to a varying extent. It is not surprising that the amœbæ are found in largest numbers in the mucus which has been discharged from the ulcers or from the irritated surface in their immediate neighbourhood. In some cases where active multiplication of amœbæ is in progress over a large surface of the bowel, and food is continually taken, the stool may be of a soft brown consistency, which on first inspection appears to differ little from the normal. It will be found, however, that there is an intimate mixture of fæcal matter and mucus in which large numbers of amœbæ occur. Sometimes the stool is more liquid and of diarrhœic nature (amœbic diarrhœa), when careful inspection will reveal small flakes of mucus in which numerous amœbæ may be found. Such cases may be due to superficial invasion of extensive areas. In many cases it is impossible to decide whether the symptoms noted are due entirely to the amœbæ, or whether they are partly the result of secondary bacterial infection of the already damaged tissues. It would be expected that an ulcerated intestine, though producing no symptoms, would be more liable than a healthy one to be irritated by food or bacteria, and if diarrhœa results from such irritation it is difficult to affirm that it is due to the amœbæ, though many may appear in the stool. It not infrequently happens that individuals who are undoubtedly infected with *E. histolytica* rarely pass amœbæ in the stools, so that many examinations have to be undertaken before an absolutely certain diagnosis can be made. In these cases inspection of the mucosa of the lower bowel by means of the sigmoidoscope has yielded valuable information. Not only can the ulcerated areas be seen, but scrapings from them will imme-

diately reveal amœbæ even in cases which have proved negative after many examinations of the fæces.

Some observers have attempted to discover a means of diagnosis in the microscopic appearance of the cells in the stools of amœbic dysentery cases, but apart from the amœbæ there is nothing characteristic of the condition. As a rule there occur a certain number of cells, including degenerating epithelial cells, macrophages which have been discharged from the ulcers, and some pus cells. They are usually present in comparatively small numbers, and it is only rarely that the stool contains the enormous number of cells usually seen in acute bacillary dysentery.

Thomson, J. G. (1918), and Acton (1918) drew attention to the frequent presence of Charcot-Leyden crystals in the stools of amœbic dysentery cases, and the latter observer concluded that their presence was pathognomonic of an infection with *E. histolytica*. Thomson, J. G., and Robertson (1921 and 1921*a*) have published an account of observations which tend to confirm the earlier conclusions. It is possible that Charcot-Leyden crystals appear in any chronic ulcerative condition of the large intestine, and that their association with *E. histolytica* is a result of the amœba being the most frequent cause of such a condition.

It has been noted above that *E. histolytica* may find its way to the liver, spleen, or even the brain, and there give rise to abscesses. In these situations the process of development is like that in the deeper tissues of the intestinal lesions. Only the large amœbæ are found, and there seems to be no tendency to the production of the small, precystic amœbæ or cysts, which have never been demonstrated in these situations.

Wherever *E. histolytica* occurs in the tissues there is no tendency for the area of invasion to be limited by the formation of fibrous tissue. On this account the abscesses of the liver are not limited by a fibrotic wall, as occurs in the case of chronic bacillary abscesses. If a section of the wall of an amœbic abscess is examined, it will be seen that there is a gradual transition from normal tissue to the completely necrotic area on the surface of the abscess wall. The amœbæ are found to be most numerous in what may be called the intermediate zone. On this account the examination of the pus which first discharges from an amœbic abscess of the liver may reveal no amœbæ. After a day or two, when apparently the surface of the abscess is breaking away and being discharged, amœbæ may appear in the discharge in large numbers. These amœbæ have the same character as the larger forms found in the intestinal ulcers.

A number of records of the presence of amœbæ in the urine have been published. In the majority of cases these are more than doubtful, but in one or two instances, as in the cases recorded by Walton (1915) and Petzetakis (1923), it seems safe to suppose that the observers were actually

PLATE I

Entamœba histolytica (x 1000) as seen in living condition in a portion of mucus from the stool of a case of amœbic dysentery. The mucus, in addition to the amœbae, contains leucocytes and red blood corpuscles. Many of the amœbae show ingested red blood corpuscles, from some of which the hæmoglobin is diffusing into the cytoplasm, giving a browinish tinge to the amœbae.

(Original.)

[To face p. 192.

dealing with amœbæ which were of the *E. histolytica* type, and not with tissue cells, which frequently lead observers astray. How the amœbæ gain access to the urine is not known, but it may be surmised that a secondary infection of the urinary tract has taken place, and that amœbæ are discharged from the lesions into the urine, where, however, they undergo degeneration more rapidly than after their discharge into the lumen of the bowel from the intestinal ulcers. There is no reason for regarding the urinary form as a species distinct from *E. histolytica*, though Baelz (1883), who was the first observer to see amœbæ in the urine, proposed the name *Amœba urogenitalis*.

Warthin (1922) observed *E. histolytica* in the vas deferens and the lumen of the dilated tubules of the epididymis and testis. The patient, a typical case of amœbic dysentery, died in spite of treatment which had cleared the intestine of its infection. The amœbæ were seen in section of the tissues. They were mostly in clots of blood and fibrin in the lumen of the dilated tubes, but in some places were invading the walls. They were remarkable in that they had phagocyted not only red blood-corpuscles, but also spermatozoa. Hines (1923) noted that a case of amœbic dysentery suffered from enlarged and extremely tender seminal vesicles. Seminal fluid expressed from the vesicles revealed typical active amœbæ with included red blood-corpuscles.

Petzetakis (1923 and 1923*b*) in Alexandria describes amœbic bronchitis in which, without actual abscess formation, the lungs appear to be in a broncho-pneumonic state. There was no evidence of liver abscess, and only certain cases gave a history of dysentery. The expectoration was said to contain active amœbæ, which in their movements, size, structure, and included red blood-corpuscles resembled *E. histolytica*. Those cases which were free from intestinal infection responded very readily to emetin treatment. It is evident that these claims require confirmation. Libert (1924) states that he obtained active forms of *E. histolytica* in a case of hepatitis by means of the duodenal tube, an observation confirmed by Boyers, Kofoid and Swezy (1925).

Several observers have recorded amœbic infections of the skin, but in most cases there is little evidence that the structures described were amœbæ at all. Maxwell (1912) observed amœbæ in fistulæ about the buttocks of cases in Formosa. In this instance it is not improbable that amœbæ had passed into the fistulæ from the intestine. Engman and Heithaus (1919) gave a description and figures of what they regarded as *E. histolytica* from ulcers on the skin of a case which was said to have an intestinal infection. Judging from the figures and description it is impossible to recognize the bodies as amœbæ, and it is evident the authors have had little experience of these organisms. Kofoid and Swezy

(1924 *a*) state, however, that they have examined the material from this case, and can confirm the occurrence of *E. histolytica* in the skin lesions. Furthermore, they claim to have seen another case showing the same infection.

Smith, S. (1924) states that he has seen amœbæ in pus from a knee-joint, while Sharp and Morrison (1925) claim to have found them in pus from abscesses in muscles.

MORPHOLOGY.—The morphology of *E. histolytica* may be considered under three headings corresponding with the three phases of development—namely, the tissue-invading form, the precystic form, and the cyst.

1. **Tissue-Invading Forms.**—These may be regarded as representing the most active phase of development (Plate I., p. 192). They occur normally in the walls of the intestinal ulcers and of the secondary lesions produced in other parts of the body. They are to be found in the fæces after discharge from the ulcers, in the pus draining from abscesses of the liver and other organs, or in material coughed up after rupture of an abscess into the lung. As has been pointed out by Dobell (1919), the amœbæ begin to degenerate soon after they have left the intestine, an explanation of the many discrepancies which characterize the accounts of the morphology of *E. histolytica* and the attempts at the establishment of new species. Even when the amœbæ are seen in perfectly fresh stools within a few minutes of their escape from the body, changes may already have occurred during their passage down the large intestine. It thus happens that in most cases in actual medical practice a diagnosis has to be made from forms which are abnormal, and which do not show the true structure of the nucleus and cytoplasm of the amœbæ as they appear in the living tissues. Such alterations in character, however, do not necessarily lead to the death of the amœbæ, for kittens may be infected by injection of material which was passed many hours before.

The tissue-invading form of *E. histolytica* as a rule varies in diameter from 20 to 30 microns, but larger or smaller forms may occur (Fig. 95, 1-4). A very characteristic feature of the amœba is its activity, large, blunt pseudopodia being formed and withdrawn in rapid succession. Progression in one direction is effected by the formation of a pseudopodium and the flowing of the entire cytoplasmic body into it. The pseudopodia are often formed quite suddenly with almost explosive violence. The remarkable activity of a group of these amœbæ when seen in a freshly passed and still warm portion of mucus can only be appreciated when seen; no description can give a satisfactory picture of this really extraordinary phenomenon. Not infrequently the amœbæ become elongated and glide in a slug-like manner over the surface of the slide without noticeable change in shape. In so doing the posterior end may have

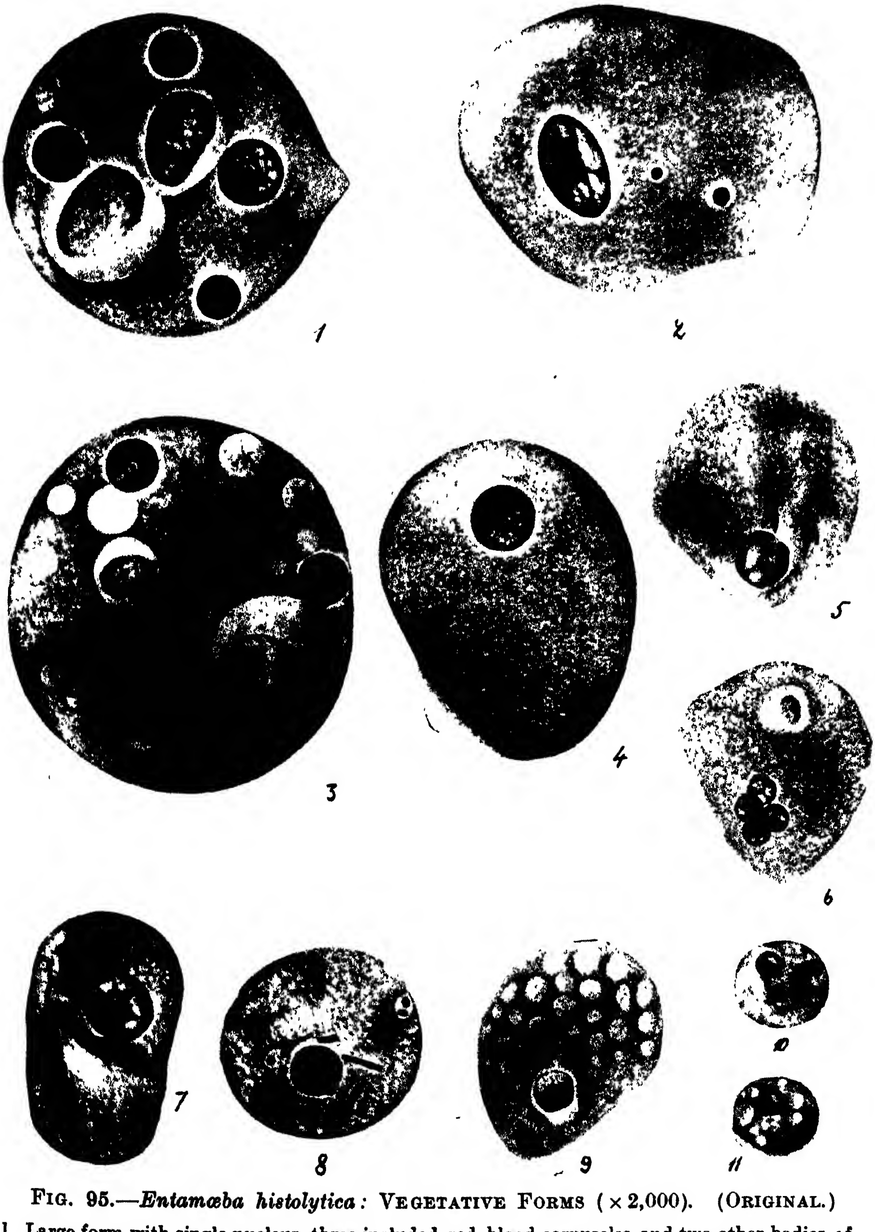


FIG. 95.—*Entamœba histolytica:* VEGETATIVE FORMS (×2,000). (ORIGINAL.)

1. Large form with single nucleus, three included red blood-corpuscles and two other bodies of doubtful nature. 2. Large form with dividing nucleus.
3. Large form with two nuclei and food inclusions, possibly altered and swollen red blood-corpuscles. 4. Large form with clear cytoplasm.
5. Form with spicular chromatoid bodies. 6. Four-nuclear forms from liver abscess pus.
7. Precystic form with large chromatoid body. 8. Precystic form with included bacteria.
9. Precystic form with clear cytoplasm. 10-11. Precystic forms of small race.

a ragged appearance, and to it bacteria and other débris may adhere. This type of movement is common in certain free-living amœbæ, and from it the name "limax" is derived. Though occasionally *E. coli* will be seen to move with an activity almost, if not quite, equal to that of *E. histolytica*, this is rarely the case, and the energetic movements of *E. histolytica* serve as one of its most important distinguishing features. When degeneration is advanced the movements become less evident, and finally cease altogether, though very often evidently degenerate amœbæ will commence moving with remarkable activity when warmed on the warm stage. In the formation of the pseudopodia the first indication is a slight elevation of the ectoplasm, but as this increases in size the endoplasm quickly flows into it. A characteristic appearance of *E. histolytica*, as seen in the stool, is that of an amœba with a clear, broad, hyaline ectoplasm sharply marked off from a granular endoplasm. Such forms may be producing pseudopodia with great activity, and these frequently consist entirely of ectoplasm. This extreme condition is probably the result of degeneration. As *E. coli* does not produce appearances of this kind, the marked ectoplasm of these altered *E. histolytica* serves as a distinguishing feature. In the perfectly fresh and normal individuals the distinction between ectoplasm and endoplasm is much less marked. The writer has examined portions of infected mucosa removed through the sigmoidoscope. Though not more than one minute had elapsed after removal, the amœbæ could be seen actively motile within the pieces of mucosa and forming ectoplasmic pseudopodia, as in the freshly passed dysenteric stool. It hardly seems possible to regard the amœbæ under these conditions as being in any way degenerate. On the other hand, the appearance of an amœba with a relatively thick ectoplasm surrounding a globular mass of granular endoplasm is undoubtedly due to a degenerative change. The endoplasm has a ground-glass appearance, and, apart from the nucleus and food vacuoles, contains, according to Dobell (1919), numerous small granules which stain *intra vitam* with neutral red. The food vacuoles include red blood-corpuscles (Fig. 95, 1), and sometimes leucocytes or other cells in various stages of degeneration (Fig. 95, 1). Sometimes the whole endoplasm appears packed with red cells, and in many cases, as these become dehæmoglobinized, the cytoplasm assumes a yellowish tint. It is only rarely that other objects are ingested by the amœbæ. The number of amœbæ in any particular specimen containing red blood-corpuscles varies considerably. Sometimes as many as 25 per cent. will show them, while in other cases a long search will reveal only a single one, or none at all. Amœbæ containing red cells may be found in stools which do not show any blood or other abnormality on naked-eye inspection.

The writer and O'Connor (1917) noted the occasional inclusion of the spores of a large bacillus, and on one occasion a large yeast-like organism. *E. histolytica* is very fastidious about the kind of food it takes up, and in the cases just mentioned, though many other structures were present in the surrounding medium apart from the spores or yeasts, all the amœbæ had selected these particular objects for ingestion. The question arises as to whether these were taken up by the amœbæ before they were discharged from the ulcers, as probably happens in the case of the included red blood-corpuscles and other cells, or whether they were ingested during the passage of the amœbæ down the large intestine. *E. coli*, on the other hand, ingests indiscriminately all kinds of objects in the intestine, but apparently not red blood-cells, so that the presence of the latter in an amœba is strong presumptive evidence of its being *E. histolytica*. Occasionally, however, undoubted *E. histolytica*, as seen in the stool, possess vacuoles with included bacteria. These organisms are sometimes seen in amœbæ in sections of ulcers from human beings and cats when the ulcer is invaded by intestinal organisms. This condition is only seen in the superficial layers. Amœbæ, both in fæces and in sections of intestinal ulcers and liver abscess, especially in cats, may contain numerous irregularly-shaped bodies which appear to be chromatoid in nature. Sometimes they bear some resemblance to Charcot-Leyden crystals (Fig. 95, 5). In the cultures of *E. histolytica*, as described by Boeck and Drbohlav (1925), the amœbæ, when grown on egg media, feed largely on bacteria. In blood media they ingest red cells also.

The nucleus of *E. histolytica* is a spherical structure 4 to 7 microns in diameter. It consists of a fine membrane enclosing an alveolar substance which in fixed material assumes the form of a network of linin threads, some of which may be radial. At the centre of the nucleus is a small karyosome surrounded by a clear area, the outer limits of which represent the inner limits of the alveolar material. In fixed specimens, again, the clear area appears in optical section to be limited by a ring of fine granules. The small karyosome is homogeneous, and is said to consist entirely of chromatin. Hartmann and others claim to have detected a centriole in the karyosome, but it is very doubtful if such a structure exists. Chromatin granules are arranged uniformly over the inner surface of the nuclear membrane. In amœbæ which have partially degenerated the nuclei may have a very different appearance. The karyosome may appear larger and be definitely excentric in position, while the chromatin on the membrane may be distributed more irregularly in the form of several larger masses. Amœbæ with nuclei which do not conform to the type are frequently encountered in perfectly fresh stools. The position of the nucleus in the endoplasm varies considerably, and is subject to constant

change, as can easily be noted by observing living amœbæ in which the nucleus can be seen. Owing to the density of the cytoplasm and its high refractive index, the delicate nucleus of *E. histolytica* is often difficult to detect in the living amœbæ. The nucleus of *E. coli*, on account of the less dense cytoplasm, is more readily seen, while structurally it is very different from that of *E. histolytica*.

E. histolytica mulitiplies in the tissues by binary fission. There is first a division of the nucleus, the details of which have been described by Dobell (1919), and this is followed by division of the cytoplasm into two more or less equal parts. Kofoid and Swezy (1924*a*, 1925) state that there are six chromosomes which appear during nuclear division (Fig. 57). Reproduction by bud formation, as described by Schaudinn (1903), and by a process of schizogony, as recorded by Job and Hirtzmann (1918), are undoubtedly the result of observations on degenerate amœbæ, or even tissue cells. On one occasion the writer has observed amœbæ with two and four nuclei in liver-abscess pus (Fig. 95, 6).

2. **Precystic Forms.**—As already explained above, under certain conditions *E. histolytica* becomes encysted, and as the cysts are smaller than the tissue-invading amœbæ, it is evident that before encystment smaller amœbæ are produced. These are probably developed from the large amœbæ by division, while the daughter amœbæ, instead of increasing in size as they do in the tissues, divide again, so that increasingly small forms are produced. It is possible that the large amœbæ, which have become more superficial in position in the intestinal lesions, suffer from a lack of fresh tissue or fluid nutriment on which to feed, so that after division growth does not take place. This shortage of food may be the stimulus which leads to encystment. The size of the amœbæ which actually encyst varies considerably, and evidence has been brought forward by the writer and O'Connor (1917), and by Dobell and Jepps (1917, 1918), that there exist definite races of *E. histolytica* which can be distinguished from one another by the average size of the cysts. In the races with small cysts these may have an average diameter of 7 microns only, while in those with larger cysts it may be as much as 18 microns. It follows, therefore, that the precystic amœbæ may vary in diameter from 7 microns upwards (Fig. 95, 7-11). There does not appear to be any evidence to support the view that in those races with small precystic amœbæ the corresponding tissue-invading forms are smaller than in those producing larger precystic amœbæ.

The precystic amœbæ have the same general structure as the tissue-invading forms, but the cytoplasm is devoid of food vacuoles, the amœbæ having ceased to ingest red blood-corpuscles or other cells, and having got rid of the remains of those taken in previously.

The precystic forms of *E. histolytica* were first seen by Elmassian (1909). He did not realize their nature, and, thinking he was dealing with a new amœba, gave it the name *E. minuta.* The name was employed subsequently by Woodcock and Penfold (1916) for the smallest races of *E. histolytica*, but Elmassian did not use it for the small race, of the existence of which he was not aware, but for the one of average size which everyone now admits is undoubtedly *E. histolytica.* Walker (1911), and Walker and Sellards (1913), appear to have been the first to realize that the small amœbæ with clear cytoplasm were the precystic forms of the large tissue-invading amœbæ. This has been amply confirmed by many observers. Shortly before encystment takes place the amœba often develops a vacuole containing glycogen, which colours brown with iodine, as well as one or more refractile bodies. The latter, which often have the form of rods with rounded ends, were named *chromatoid bodies* by Dobell. They show no marked affinity for iodine, but stain black with iron hæmatoxylin. It is very improbable that they are chromatic in nature. They have well-defined edges, and are readily seen as greenish refractile bars in the living amœbæ or cysts. The margin of the glycogenic vacuole, as stained with iodine, is not sharply defined, for it gradually shades off into the surrounding cytoplasm. In the case of the cysts of *Iodamœba bütschlii*, the substance in the vacuole is much denser than that in the vacuole of the cysts of *E. histolytica*, and in iodine-stained specimens the limits of the vacuole, or more correctly those of the glycogenic body within it, are very sharply defined, the brown colour of the included substance ceasing abruptly at the margin of the vacuole (Plate II., 5, 6, 9, and 11-14, p. 250).

The nuclei of the precystic amœbæ resemble those of the tissue-invading forms, except that the chromatin on the membrane often occurs in larger masses. In some cases the nuclei possess a single large crescentic mass in addition to smaller ones. Dobell (1919) states that chromatin granules occur also on the linin network, a condition which he does not find in the normal nuclei of the tissue-invading forms.

The precystic amœbæ are not so active as the tissue-invading forms, and on account of the larger chromatin granules of the nuclei they may be difficult to distinguish from the corresponding stages of *E. coli.* In these cases it will be necessary to discover the characteristic cysts. The smaller races are still more difficult to distinguish, as they may be confused with *Endolimax nana.* The structure of the nucleus, as seen in stained preparations, is important, and a final diagnosis may not be possible till cysts have been found, it may be after repeated examinations on different days.

The precystic amœbæ and the cysts of *E. histolytica* were first

accurately studied by Huber (1903), though the cysts had previously been seen and figured by Quincke and Roos (1893), and Roos (1894). They were again seen by Viereck (1907), and by Hartmann and Prowazek (1907), who regarded them as belonging to distinct species of amœbæ, which were named *E. africana* and *E. tetragena* respectively. This supposed difference, however, was the outcome of Schaudinn's erroneous account of the development of *E. histolytica*, which was almost entirely based on the appearances seen in degenerating amœbæ. There can be no doubt that *E. africana*, *E. tetragena*, and *E. minuta* are merely forms of *E. histolytica.* The small amœba described by Prowazek (1912*a*) as *E. hartmanni*, and by Kuenen and Swellengrebel (1917) as *E. tenuis*, is undoubtedly a small race of *E. histolytica*, producing cysts 6 to 8 microns in diameter.

3. **Cyst.**—The cyst which is formed round a precystic amœba seems to be composed at first of a soft material which quickly shrinks and hardens to a resistant, colourless, smooth, transparent capsule. It is completely filled by the cytoplasm of the amœba (Fig. 96). The cyst wall is about 0·5 micron in thickness, its inner and outer margin being visible in optical section. When first formed, it encloses the amœba and the structures it contains. Thus, the newly-formed cyst contains the cytoplasm and nucleus, and also the vacuole and chromatoid bodies if these happened to be present in the amœba. The cysts are generally spherical, but they may be elongated or even dumb-bell-shaped. Within the cyst the single nucleus divides to form two nuclei, and these divide again, so that in the mature cyst four nuclei are present (Fig. 57). Very frequently the four nuclei are arranged in pairs at opposite sides of the cyst. On very rare occasions eight nuclei may be found. According to Dobell (1919) the vacuole, if present, gradually disappears as the cyst develops. It appears as if the glycogen of the vacuole is used up during the nuclear divisions. The chromatoid bodies are similarly absorbed while the cyst is waiting outside the body to be ingested by a new host. The chromatoid bodies usually have the form of rods with rounded ends, and very commonly one, two, or three are present in the cyst. They vary in length from 5 to 10 microns, but longer or shorter forms may occur. Sometimes they are of a different shape, and may be more rounded or irregular in outline. On other occasions they are filamentous structures, or a large number of small, irregularly-shaped bodies may be present. When seen in the living cyst they appear as homogeneous structures which have a refractive index higher than that of the rest of the cyst. On this account they are readily distinguished. The chromatoid bodies are of great diagnostic value, for they occur much more rarely in the cysts of *E. coli*, in which case the eight nuclei characteristic of the mature cysts of this amœba will be noted.

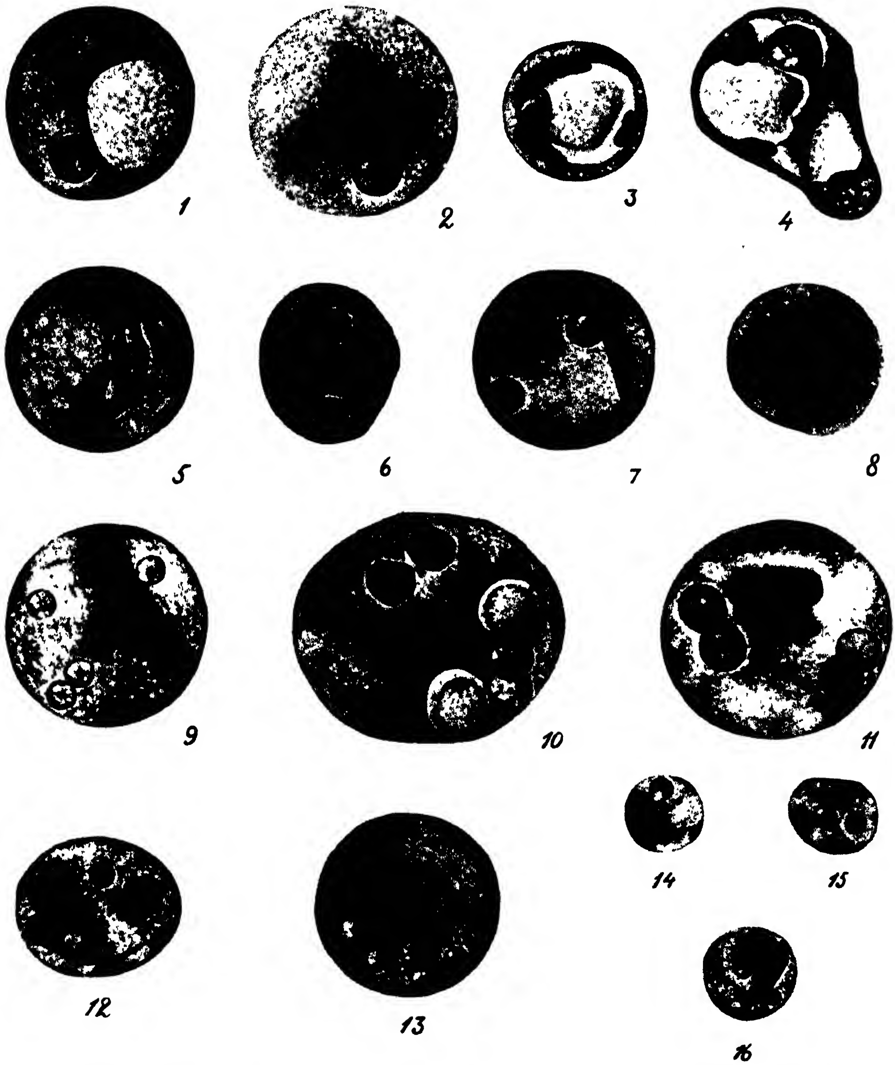


FIG. 96.—*Entamœba histolytica:* ENCYSTED FORMS (× 2,000). (ORIGINAL.)

1. Form with one nucleus and vacuole. 2. Form with one nucleus and chromatoid bodies.
3. Form with one nucleus, large vacuole, and chromatoid bodies.
4. Irregularly shaped form with single nucleus, large vacuole, and chromatoid bodies.
5. Form with dividing nucleus.
6. Binucleated form without vacuole or chromatoid bodies.
7. Binucleated form with chromatoid bodies.
8. Binucleated form with numerous chromatoid bodies.
9. Form with four nuclei and chromatoid bodies.
10. Form with four nuclei, chromatoid bodies, and vacuoles with inclusions.
11. Form with four nuclei and two chromatoid bodies. 12. Form with four nuclei alone.
13. Form with six nuclei, two of the original four having divided. Similarly, forms with eight nuclei occasionally occur. 14-16. Forms belonging to a small race.

The cyst of *E. histolytica*, when seen in fresh material, has a greenish refractile appearance. Owing to its refractiveness, which is much more marked than that of the cysts of *E. coli*, it is sometimes very difficult to distinguish the nuclei, though the chromatoid bodies may be easily seen. In iodine solution, however, all the contents can be clearly distinguished (Plate II., 5-10, p. 250).

The cysts of *E. histolytica* vary in diameter from 5 to 20 microns according to the particular race, but all the cysts of any one race are not of the same size. Thus, in six cases studied by the writer and O'Connor (1917) the diameter of the cysts varied as follows: 7 to 9 microns, 7 to 11 microns, 10 to 13 microns, 10 to 14 microns, 11 to 15 microns, 12 to 18 microns. The cysts remained constant in their average size during the observation, which in some cases extended over several months, so that it would appear that true races are represented (Fig. 10). Some observers, however, believe that the small cysts belong to a distinct species of amœba. Prowazek (1912 *a*) gave the name *E. hartmanni* to these forms, Kuenen and Swellengrebel (1917) the name *E. tenuis*, and Brug (1917) the name *E. minutissima*. Though the writer has repeatedly observed the appearance of cysts in the stools of cases in which the acute symptoms of amœbic dysentery were subsiding, these have always been of the average size, or larger than this. In no case has he seen the small cysts appear under these circumstances. It cannot be regarded as finally established that the races of *E. histolytica* which produce the small cysts are able to give rise to amœbic dysentery. Drbohlav (1925*b*) has cultivated a small race. In the cultures the amœbæ resembled the typical *E. histolytica*. They did not, however, ingest red blood-corpuscles, and though producing infection in kittens, failed to give rise to dysentery and ulceration of the large intestine. The precystic amœbæ as seen in fæces correspond in size with the cysts, so that they are smallest in those races which produce the smallest cysts. There are no data, however, to show whether a corresponding variation in average size of the tissue-invading forms occurs. Shimura (1918) described a race of *E. histolytica* with small cysts as a non-pathogenic race, but it has to be remembered that the majority of carriers who show no symptoms are passing cysts of the average size. If carriers alone were examined, the average-sized cysts might with equal justification be regarded as belonging to non-pathogenic races. In the case of the smaller-sized cysts, diagnosis from living specimens may be very difficult unless the characteristic rod-like chromatoid bodies are present. In iodine the details are much clearer, but it is often necessary to prepare stained films before making a final diagnosis.

The cysts passed from the body may contain one, two, or four nuclei.

It sometimes happens that the majority of the cysts seen in a specimen of fæces are in the uninucleate condition, while two and four nuclear specimens may be very difficult to find. In other cases the majority of cysts have four nuclei. There seems to be no regularity regarding the stage of development in which cysts are passed, and this is not surprising when it is remembered that the evacuation of the large intestine depends upon the host, and may occur either before or after encystment has commenced.

The cysts of *E. histolytica* will remain alive for a considerable time after leaving the body in fæcal matter, but if brought into clean water they will survive a much longer period. During this time the chromatoid bodies gradually disappear. When the cysts die, various degenerative appearances become evident, and it is found that the cysts will stain immediately if brought into eosin solution. It appears that eosin solution may be used as a test of the life of a cyst, as also of the free amœbæ themselves. The live cysts or amœbæ will not stain immediately, whereas the dead ones will become red at once. This can readily be demonstrated by watching the effect of eosin on cysts before and after heating to a temperature sufficient to kill them.

In his description of the development of *E. histolytica*, Schaudinn (1903) described a method of reproduction by bud formation. The nucleus was supposed to give off chromatin material into the cytoplasm in the form of granules, which collected in groups on the surface of the amœbæ. Small cytoplasmic buds, each containing a group of chromatin granules, were formed. These buds were described as becoming enclosed in very resistant capsules, forming spores, which were much smaller than the cysts of *E. histolytica*, as they are now known. Schaudinn claimed to have produced infection in cats by means of these spores after complete drying, a procedure which is known to kill immediately the cysts of *E. histolytica*. Recent investigations have failed entirely to confirm Schaudinn's statements, so that it is safe to conclude that the budding process and spore formation as described by him do not take place.

PATHOGENICITY.—It has usually been assumed that any individual who is harbouring *E. histolytica* must have definite lesions of the intestinal wall, in the tissue of which the amœbæ are living, but though this may be true to a very large extent, the successful culture of the amœbæ in tissue-free media suggests that they may sometimes live on the surface of the intestine without giving rise to actual lesions. In some cases the lesions give rise to large quantities of blood and mucus, so that the acute condition of amœbic dysentery results. In the great majority of cases, however, very few, if any, symptoms are noted, so that the infection can only be detected by microscopic examination of the fæces. The fact that in some individuals the symptoms of acute dysentery occur, while

in others the infection is of a mild nature, may be comparable with what is known to occur in bacterial infections. Many individuals harbour pathogenic bacilli in their throats without having symptoms of the disease which may be caused by these organisms. Invasion of the tissues may occur because the resistance of the host is lowered or because the virulence of the organism is increased. The former seems to be the most rational explanation, and in the case of *E. histolytica* infections it would seem that the resistance of the intestine is lowered from time to time, with the result that active multiplication of the amœbæ with their extension into the tissues takes place, so that acute dysentery supervenes. On the other hand, it has to be remembered that the virulence of protozoa may vary considerably. In the case of trypanosomes it is well known that passage of a strain from one animal to another may so change it that it will bring about death in a few days instead of a few months. It is possible that the virulence of *E. histolytica* may vary in a similar manner, but there is no evidence that this occurs. By quick passage of a strain from one man to another the virulence might be so increased that eventually every individual infected would acquire an acute and fatal amœbic dysentery. Whether this actually happens in nature is not known, but judging from the results of experiments on kittens the writer can find no reason to suppose that the amœbæ from carrier cases with few or no symptoms are less virulent than those from acute cases. Brumpt (1925), however, suggests that there exist two types of amœba included under the name *E. histolytica*, the one infective to kittens and the other not. To the latter he gives the name *Entamœba dispar*, and suggests that it accounts for many of the carrier cases in countries where amœbic dysentery is uncommon. The writer does not believe that physiological data of this kind afford a means of distinguishing species.

SUSCEPTIBILITY OF ANIMALS.—Lösch (1875) succeeded in infecting a dog with *E. histolytica*, and this experiment was repeated by Hlava (1887), Kruse and Pasquale (1894), Harris (1901), and Dale and Dobell (1917). Young cats, however, are more easily infected, and it is with them that most experimental work has been done. Hlava (1887) was the first to produce infection in these animals, and he was followed by Kartulis (1891), Kovács (1892), Quincke and Roos (1893), Kruse and Pasquale (1894), Marchoux (1899), the writer (1912*d*), and many others. Kruse and Pasquale also infected cats with the amœbæ obtained from liver abscess. The infection has generally been produced by injections of dysenteric stools *per anum*, and this is the most reliable method for infecting these animals. Unless cysts are present the animals cannot be infected by feeding, a fact first demonstrated by Quincke and Roos (1893), the first observers to describe the cysts of *E. histolytica*. Huber

(1903), who rediscovered the cysts, confirmed this observation, which was repeated by Kuenen and Swellengrebel (1913), the writer and O'Connor (1917), and Dobell (1917), and others. The infection in kittens is, as a rule, of a very severe type, the whole of the surface of the large intestine being infected with amœbæ. The infection usually commences at the lower part of the large intestine, and it is here that the changes in the mucosa are most marked. Sellards and Leiva (1923*a*) have shown that this is probably due to the natural stasis which occurs at this point. By ligaturing the large intestine of cats at various levels and inoculating infective material directly into the cæcum they have demonstrated that the infection commences and is most marked just above the ligature. They see in this an explanation of the fact that in human beings amœbic ulceration is most marked at the point where stasis occurs. If the animals live long enough, definite ulcers occur as in human beings, but frequently all the glands are infected over the whole gut wall and death results from the general necrosis of the mucosa which is set up. Sellards and Leiva (1923*a*) have shown that bacterial invasion of the blood also plays a part, for they have cultivated various intestinal organisms from the blood of infected cats. As a method of diagnosis of amœbic infection in the cat they have employed a daily saline enema. The fluid is quickly returned, and the flakes of blood-stained mucus which it carries with it can be examined for amœbæ. Infected cats frequently pass *per anum* a whitish fluid containing many broken-down cells and enormous numbers of amœbæ. In less acute cases the stools resemble those of amœbic dysentery in man, there being fæcal material containing masses of mucus stained with dark red blood. Recovery rarely takes place in cats, and when it does the infection dies out, there being no carrier condition corresponding to that in human beings. The cysts of *E. histolytica* are never formed in cats. As in man, secondary infection of the liver may take place, leading to the formation of liver abscess, an observation which was first made by Marchoux (1899), and subsequently by Craig (1905), Werner (1908), Huber (1909), the writer (1912*d*), Dale and Dobell (1917), Mayer (1919), and Sellards and Leiva (1923*a*). Harris (1901) noted a similar condition in an experimentally infected dog.

The infection in cats may be maintained indefinitely by injecting the intestinal contents *per rectum* from one animal to another. The cats must be only a few weeks old, as large, full-grown animals are more resistant to infection. As the cysts of *E. histolytica* never occur in cats, the infection cannot be handed on from cat to cat by feeding with intestinal contents. The writer has never succeeded in infecting kittens by means of material from liver abscess, in spite of the presence of active amœbæ.

Guinea-pigs have been infected by Baetjer and Sellards (1914), and

by Chatton (1917, 1918*d*). This may be accomplished by injections *per anum* or by feeding with cysts *per os*. In these animals dysenteric symptoms do not appear, but large tumours develop about the cæcum, and these are found to consist of overgrowths of the tissues due to the amœbæ, which grow and multiply within them. Huber (1909) claims to have produced a chronic ulceration of the cæcum in rabbits by feeding them with cysts, while Lynch (1915) and Brug (1919*a*) claim to have infected rats. Kessel (1923*a*) states that he has infected rats and mice with *E. histolytica*. He finds (1923) that natural amœbic infections of these animals can be excluded by the examination on two successive days of fæces obtained after the administration of a purge in the form of stale bread soaked in magnesium sulphate solution. To such animals cysts of *E. histolytica* were given. The infections produced are of a chronic nature, and persist for months. Free forms, as well as characteristic cysts, could be obtained in the fæces of the animals after giving them magnesium sulphate. The infection was handed on from rat to rat. Chiang (1925) has also infected rats. The amœbæ from the experimental rats, as well as a naturally occurring rat strain (*E. histolytica* var. *murina*), gave rise to typical infections when inoculated to kittens. Clean rats kept with infected ones contracted an *E. histolytica* infection.

Attempts which have been made to infect monkeys have been inconclusive, owing to the fact that these animals are liable to natural amœbic infections due to two species of amœbæ which are very similar to *E. histolytica* and *E. coli*. These animals suffer from amœbic dysentery, and even amœbic abscess of the liver, as pointed out by Eichhorn and Gallagher (1916) and others (see p. 226).

CULTIVATION.—Many attempts have been made to cultivate *E. histolytica* in artificial media, but the only successful results are those of Cutler (1918) and Boeck and Drbohlav (1925). Other observers have cultivated only coprozoic amœbæ. Cutler used two media.

The first was made as follows: The entire contents of an egg were broken up by shaking in a glass bottle with beads. To the broken-up egg 300 c.c. of distilled water were added, and mixture was effected by shaking. The fluid was then brought gradually to the boiling-point in a water bath, and kept at this temperature for half an hour. During the heating the mixture was shaken, so that a fluid was obtained in which minute egg particles were suspended. It was then distributed in quantities of 5 c.c. in test-tubes and autoclaved. Before use a few drops of blood were added to each tube.

The second medium was prepared by boiling 500 c.c. of human blood-clot for an hour in a litre of water. To the filtrate was added 0·5 per cent. sodium chloride and 1 per cent. peptone. The fluid was then tubed

and sterilized by steaming for twenty minutes on three successive days. As in the case of the egg medium, a few drops of blood are added before inoculation.

Attempts were made to cultivate amœbæ from forty-five samples of fæces containing *E. histolytica*, and amœbæ were grown from six which contained blood and mucus. Bacteria grew in the media as well as the amœbæ, and it was necessary to subculture every twenty-four to seventy-two hours on account of the quantity of acid produced by the bacteria. A temperature of 28° to 30° C. was better than a higher one, as bacterial growth was reduced. Subculture was effected by transfer of 0·5 to 1 c.c. of the culture. By this means cultures were maintained for over three months, and not only did multiplication of the amœbæ take place, but encystment also occurred. Cats were infected by inoculation *per rectum* with cultures of more than two and a half months' standing, and typical dysenteric symptoms with amœbæ resulted, while post-mortem examination showed the characteristic amœbic lesions, from which fresh culture was obtained. Other animals were infected by feeding them on cultures containing cysts. Dobell (1919) stated that he attempted without success to cultivate *E. histolytica* by this method, and concluded that there must have been some fallacy in Cutler's work. The writer also failed to repeat Cutler's experiments. Barret and Smith (1923, 1924), however, obtained cultures of another amœba, *Entamœba barreti* of the turtle, *Chelydra serpentina*. The medium used was a mixture of human blood-serum 1 part and 0·5 per cent. sodium chloride solution 9 parts. In each tube 5 c.c. of the mixture was used. A small quantity of mucus obtained from the intestinal wall was inoculated at the bottom of the tubes, which were kept at 10° to 15° C., or at room temperature. At first it was necessary to subculture every twenty-four or forty-eight hours, but when a culture was established a weekly transfer was sufficient. Two strains were kept for nine months, during which thirty subcultures were made. The amœbæ multiplied actively, and corresponded in every way with those seen in the intestine of the turtles. No cysts were found, however. Cultures of *E. ranarum* of the frog have also been obtained. These results, which were obtained with amœbæ of cold-blooded hosts, led Barret and Smith to suggest that Cutler may have been more successful with *E. histolytica* than some had supposed.

Quite recently Boeck and Drbohlav (1925) have cultivated *E. histolytica* on solid egg and blood agar slopes covered with Locke's solution containing serum or egg albumin. From two human cases *E. histolytica* was isolated and maintained in subculture for many generations, in one case for more than eight months, during which 150 subcultures were made. Subculture was made every two or three days, and the tubes were kept at

30° to 37° C. Bacteria were constantly present in the amœbæ. In the blood medium red blood-corpuscles were frequently ingested by the amœbæ, which structurally corresponded with *E. histolytica*. Even after as many as ninety-three subcultures kittens could be infected with the cultural forms, and a condition exactly like that arising from the injection of material from cases of amœbic dysentery resulted. In a few instances the animals developed amœbic abscess of the liver. Cultures were also obtained from the infected kittens. On one occasion cysts were observed in the culture tubes. Drbohlav (1925*a*) has repeated these experiments, which have also been confirmed by Thomson, J. G. and Robertson (1925).

ABERRANT FORMS OF E. HISTOLYTICA.—Working in North China, Faust (1923) has observed in four cases of dysentery a peculiar type of amœba which ingests not only red blood-corpuscles, but also bacteria (Fig. 97). The characteristic feature of the organism, which has a diameter of 16 to 17 microns when quiescent and globular, is its posterior end. When active it is definitely elongated, with a rounded anterior end and a tapering posterior end which terminates in a pointed protoplasmic structure (caudostyle), surrounding which are sometimes several smaller protoplasmic projections. Débris tends to become adherent to the region of the caudostyle. The nucleus, measuring 3 to 4·5 microns in diameter, is always situated in the rounded anterior end of the organism. On the inner surface of the nuclear membrane are minute chromatin granules. The karyosome is a star-shaped structure which may have a central vacuole. The rays consist of chromatin granules. In two of the cases examined the infection was a pure one, while in the other two cases *E. histolytica* occurred in one and *E. coli* in the other. Faust states that there was no difficulty in distinguishing these amœbæ from other species. Though the cases were followed for some time, no encysted stages of the organism were seen. The amœba appears to fix and stain badly, as compared with *E. histolytica* or *E. coli*, which sometimes occurred in the same sample of fæces. Owing to the features described above, Faust places the amœba in a new genus as *Caudamœba sinensis*. He believes that it is a cause of amœbic dysentery. As regards the validity of this

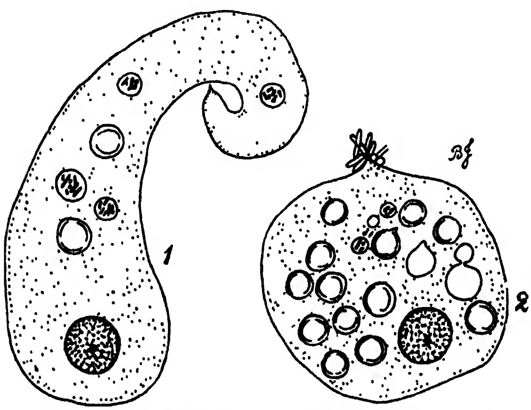


FIG. 97.—"*Caudamœba sinensis*" FROM THE HUMAN INTESTINE (× 2,000). (AFTER FAUST, 1923.)

species it is difficult to form an opinion, as the encysted stages were not seen. In any case there seems to be little ground for the creation of a new genus. It has to be remembered, however, that undoubted *E. histolytica* often move in a slug-like manner, as noted by Dobell and O'Connor (1921), and that many free-living amœbæ, as well as *E. histolytica,* may develop the slug-like form with tapering posterior end to which débris adheres, while other amœbæ in the same pure culture move in the more normal amœboid manner. Whether *Caudamœba sinensis* is actually distinct from *E. histolytica* future investigations alone will show, but it seems to the writer that sufficient evidence to justify the distinction has not yet been produced. Recently the writer has had an opportunity of observing *E. histolytica* in cultures. The assumption of a slug-like form with tapering posterior end to which débris adheres is quite common. The fact that bacteria as well as red blood-corpuscles occurred in vacuoles is a feature which may be met with in undoubted *E. histolytica.* Schubotz (1905) has figured an elongated form of *E. blattœ* of the cockroach which bears some resemblance to *C. sinensis*, while Jepps (1923) has described a somewhat similar form of *E. gingivalis,* and Keilin (1917) one in *E. mesnili* (Fig. 109).

Chaterjee (1920) gave the name *Entamœba paradysenterica* to amœbæ which he found post-mortem in dysenteric lesions, and which he regarded as a distinct species on account of certain peculiarities of nuclear structure. The writer has seen preparations of this amœba, which is unquestionably a degenerate *E. histolytica.*

Kofoid and Swezy (1924*b*) gave the name *Karyamœba falcata* to an amœba of the human intestine. As the generic name was preoccupied, they (1925*a*) changed it to *Karyamœbina* (Fig. 98). The amœba was first described from three cases. The first harboured, in addition, *E. histolytica, E. coli, Endolimax nana, Dientamœba fragilis*, as well as the form described as *Councilmania lafleuri ;* the second *E. histolytica ;* and the third *E. histolytica, E. coli*, and *C. lafleuri.* Three further cases were reported in their second paper. The chief distinguishing feature is the nucleus and the method of nuclear division. The nucleus has a definite membrane, upon which the chromatin is massed in one or two, rarely more, crescentic clumps. There is an excentric karyosome round which is a halo. In division the nucleus elongates, and there is formed at each end a deeply staining pole cap. On this account the amœba is supposed to be allied to members of the genus *Vahlkampfia* (see p. 177). In *Vahlkampfia,* however, the pole caps are formed from the divided karyosome, and it is definitely stated that in *K. falcata* the karyosome does not divide. In this form the pole caps are merely terminal aggregations of the large chromatin masses on the nuclear membrane. On this account the amœba cannot be allied with *Vahlkampfia.* It is said that in *K. falcata* about twenty chromosomes occur at the equator of the elongating nucleus. Cysts have not been observed.

As pointed out by the writer (1925), from the fact that the cases from which *K. falcata* was first recorded harboured *E. histolytica* also, while two of them had other amœbæ as well, it seems that definite proof that the so-called *K. falcata* is a distinct entity has not been produced. It is known that in *E. histolytica* the nucleus not infrequently shows chromatin arranged in crescentic masses, and it has yet to be demonstrated that in nuclear division such nuclei never assume the form supposed to be characteristic of *K. falcata.*

Of quite another nature are the supposed amœbæ which Kofoid and Swezy (1922) and Kofoid, Boyers and Swezy (1922) have described from the

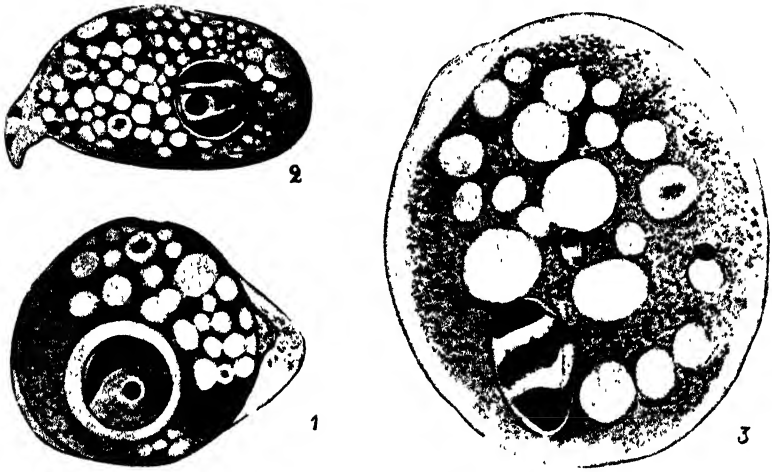


FIG. 98.—FREE FORMS OF "*Karyamœbina falcata*" (× 2,000). (AFTER KOFOID AND SWEZY, 1924, SLIGHTLY REDUCED.)

1. Form with clear pseudopodium and single crescentic body on nuclear membrane.
2. Nucleus with two crescentic bodies united by a fibre.
3. Dividing form: nucleus with pole caps, centrioles united by centrodesmose, and equatorial plate of about twenty dividing chromosomes.

bone marrow of cases of arthritis deformans, and from the hypertrophied lymphatic glands in Hodgkin's disease. Because of a particular type of division exhibited by the nuclei of certain cells, it is concluded that they are not only amœbæ, but actually *E. histolytica.* It must be apparent to most protozoologists that far more convincing evidence is required before this view can be accepted.

(*b*) Non-Pathogenic Forms.

Entamœba coli (Grassi, 1879) Casagrandi and Barbagallo, 1895.—**Chief synonyms**: "Amœbæ" Lewis, 1870; "Amœbæ" Cunningham, 1871; *Amœba coli* Grassi, 1879; "Amœba coli mitis" Quincke and Roos, 1893; "Amœba intestini vulgaris" Quincke and Roos, 1893; *Entamœba coli* Casagrandi and Barbagallo, 1895;

Entamœba hominis Casagrandi and Barbagallo, 1897; *Entamœba coli* Schaudinn, 1903; *Amœba coli* Brumpt, 1910; *Entamœba williamsi* Prowazek, 1911; *Entamœba hartmanni* Prowazek, 1912 (*pro parte*); *Entamœba brasiliensis* Aragão, 1912 (*pro parte*); *Löschia coli* Chatton and Lalung-Bonnaire, 1912 ; *Entamœba coli communis* Knowles and Cole, 1917 (*pro parte*); *Endameba intestinivulgaris* Aragão, 1917 ; *Endameba coli* Craig, 1917; *Endameba hominis* Pestana, 1917 ; *Councilmania lafleuri* Kofoid and Swezy, 1921.

This amœba is a harmless commensal of the digestive tract of man, and is in no sense a tissue-invading amœba like *E. histolytica.* According to Dobell, it was first seen by Lewis (1870) in India, and was described more accurately by Cunningham (1871). Grassi (1879–1888) gave various descriptions of the organism, and erroneously believing it to be identical with the form originally studied in dysenteric cases by Lösch (1875), gave it the name *Amœba coli*, a name which should have been employed for the pathogenic form only. As has been explained above, Schaudinn again committed this error, and though, according to the strict laws of nomenclature, *E. coli* should be the name of the pathogenic amœba, its employment in this sense would lead to endless confusion, so that it is better to retain the name *E. coli* for the harmless amœba. Grassi realized that the amœba was a harmless inhabitant of the human digestive tract, for he found it not only in sick, but also in healthy people. Quincke and Roos (1893) gave a good description of *E. coli*, which they distinguished from *E. histolytica*, while Casagrandi and Barbagallo (1895, 1897) studied the same organism, which they named *Entamœba coli.* They took a retrograde step in assuming that this was the only form which occurred in healthy as well as in dysenteric subjects. Schaudinn (1903) clearly stated that there were two amœbæ, the one a tissue-invading form and the other a harmless commensal, and his reputation as a protozoologist resulted in a universal acceptance of this view, which had been previously put forward by Quincke and Roos. Since Schaudinn's time numerous names have been given to amœbæ which are undoubtedly merely forms of *E. coli.* These have been fully discussed by Dobell (1919), and it is unnecessary to enter into the matter here.

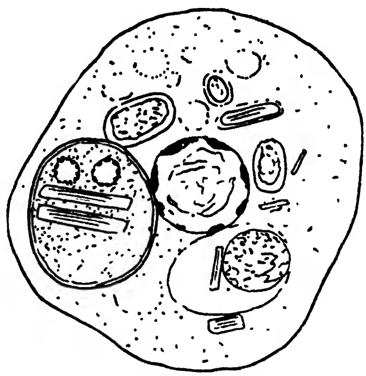


FIG. 99.—*Entamœba coli* WITH INGESTED CYST OF *E. histolytica* (× *ca.* 2,000). (AFTER WENYON AND O'CONNOR, 1917.)

Entamœba coli is a very common parasite of the human intestine. In tropical lands, or in other countries where sanitary arrangements are not satisfactory, it is probable that no person escapes infection. Like *E. histo-*

lytica it lives in the large intestine, but it does not invade the tissues. It develops in the intestinal contents, especially on the surface of the mucosa, where it feeds on bacteria, yeasts, and other material. It will ingest cysts of other Protozoa, such as those of *Giardia* and *Isospora*, and even the cysts of *E. histolytica* (Fig. 99). It does not appear to ingest red blood-corpuscles in its natural habitat. In cases of bacillary dysentery, when enormous numbers of red cells occur in the stool, *E. coli* may sometimes be seen moving about amongst them, and showing no inclination to take them in. The writer has seen red blood-corpuscles adhering to the surface of motile *E. coli*, which, however, showed no tendency to engulf them. Lynch (1924) has, however, been able to induce *E. coli* to ingest red cells by incubating them with blood in a test-tube. In the writer's experience this never occurs in the intestine, and, if it does, it must be such a rare phenomenon that the general rule given above, that an amœba with included red cells is almost certainly *E. histolytica*, still holds for all practical purposes. Like *E. histolytica*, *E. coli* becomes encysted in transparent resistant cysts, and it is these forms which spread infection from one individual to another.

MORPHOLOGY.—*E. coli* may be considered in three stages: the adult form, the precystic form, and the cyst.

1. **Adult Form.**—The fully-grown *E. coli* (Fig. 100) is on an average larger than *E. histolytica*, and as usually seen it has a diameter of 15 to 30 microns. Occasionally very much smaller forms, under 10 microns in diameter, occur. Generally, the amœbæ are much less active than *E. histolytica*, the movements being very sluggish. Occasionally, however, the writer has seen undoubted forms of *E. coli* moving with a rapidity comparable with that of *E. histolytica*. The ectoplasm is not so clearly defined as in *E. histolytica*, and in the normal individual there is merely a superficial layer which is clearer than the endoplasm into which it merges. The degenerating forms of *E. coli* do not show the exaggerated extension of ectoplasm which is such a characteristic feature of the abnormal forms of *E. histolytica*. The endoplasm of *E. coli* is often extensively vacuolated, and the vacuoles contain a great variety of objects which are chiefly bacteria. The general appearance of the amœba is that of a slightly greyish object, which contrasts with the greenish tint resulting from the high refractive index of the denser *E. histolytica*. *E. coli* is much more fluid in consistency than *E. histolytica*. Sometimes the amœbæ show various fissures or rectangular vacuoles, which are probably the result of degenerative changes.

1-3. Forms with vacuolated cytoplasm, including bacteria. 4. Binucleated form.
5-6. Forms with irregularly shaped nuclei and very coarse chromatin masses.
7. Form which has ingested a small binucleated cyst.
8. Form showing excentric position of karyosome.
9-10. Small individuals. 11. Large precystic form with clear cytoplasm.

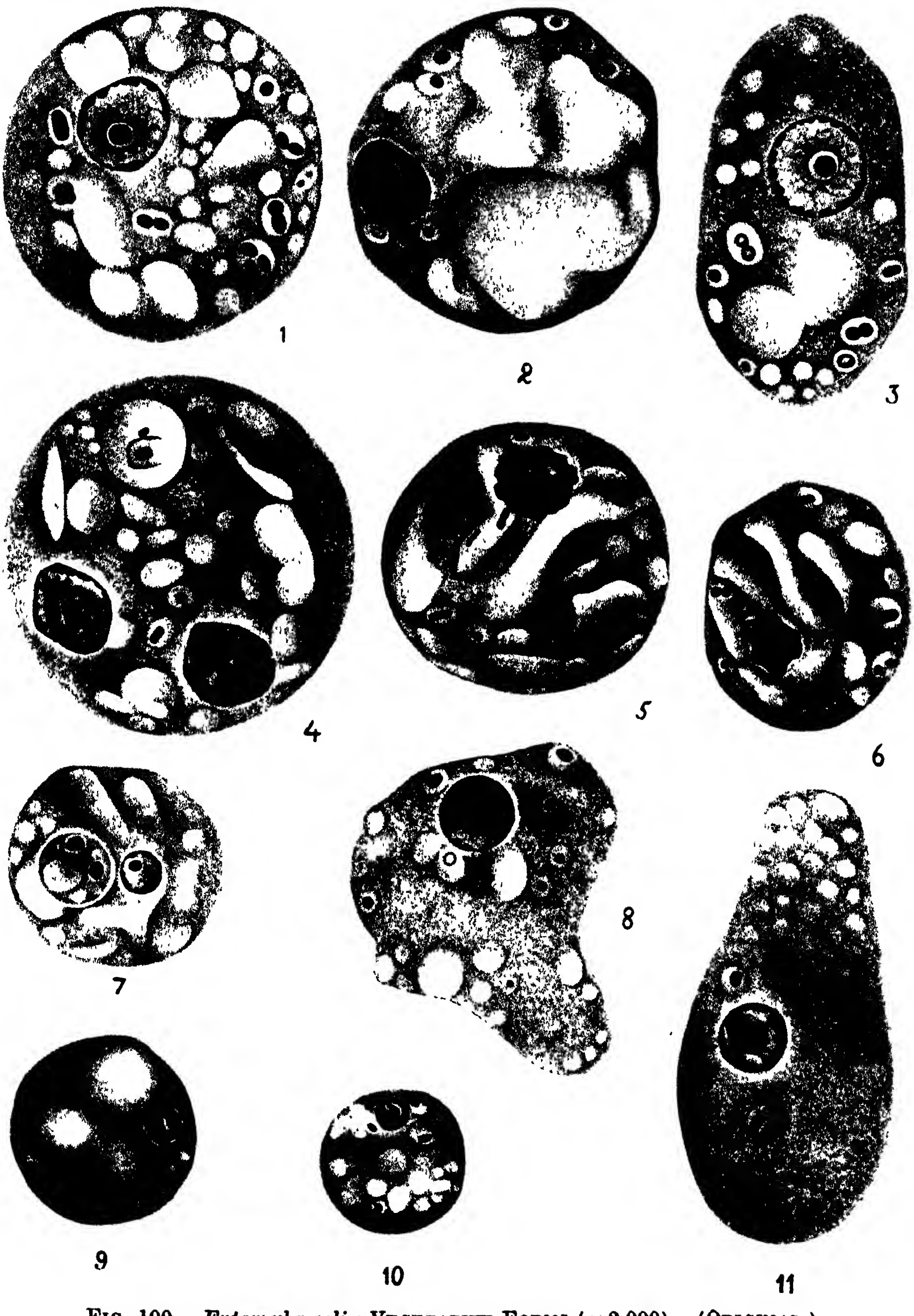


FIG. 100.—*Entamœba coli* : VEGETATIVE FORMS (× 2,000). (ORIGINAL.)

[*For description see opposite page.*

The nucleus of *E. coli* is a larger and coarser structure than that of *E. histolytica*, and is readily distinguished in the living amœba on account of the low refractive index of the cytoplasm. In stained specimens it is seen to have a thicker membrane than the nucleus of *E. histolytica*. The chromatin granules are coarser and the karyosome, when it is a single compact granule, is larger, as also is the clear area around the karyosome. Dobell (1919) states that the karyosome is nearly always excentric, and that chromatin granules occur on the linin network between the clear area and the nuclear membrane. The nucleus of *E. coli* thus differs from that of *E. histolytica* chiefly in its coarseness, and as the nucleus of *E. histolytica* quickly changes in character as a result of degeneration, it is very frequently impossible to distinguish the two amœbæ as they occur in the stool from the appearance of their nuclei alone. The presence of a larger number of food vacuoles containing bacteria and other objects is a more reliable means of recognizing *E. coli*. It must be admitted, however, that it is very often impossible to distinguish between *E. coli* and *E. histolytica* in the free condition. In such cases search must be made for the characteristic cysts. *E. coli* reproduces by binary fission, like *E. histolytica*. The details of nuclear division have not been followed completely in the free forms; they are very similar to those of *E. histolytica*. During the division of nuclei in the cysts Swezy (1922) states that there are probably six chromosomes. Several observers, including Schaudinn (1903), Casagrandi and Barbagallo (1897), and Mathis and Mercier (1917), have described a process of schizogony of *E. coli*. In stained films it is often very difficult to detect the wall of a cyst, which becomes highly transparent in cleared preparations. If such a cyst has an irregular shape, as is not infrequent in preparations, the appearance of an amœba with eight nuclei is produced. The writer has seen and marked such forms as possible schizogony or multinucleate stages, but in all cases it has appeared more probable that they were distorted or irregularly shaped forms which were really encysted. There seems no reason to suppose that *E. coli* in the free condition reproduces in any other way than by binary fission.

2. **Precystic Forms.**—As in the case of *E. histolytica*, prior to encystment there are produced amœbæ which are smaller than the adult forms and have a cytoplasm cleared of all food materials (Fig. 100, 11). The precystic forms of *E. coli* are very similar to those of *E. histolytica*, but as the average size of the cyst of *E. coli* is greater than that of *E. histolytica*, so the precystic amœbæ are correspondingly larger. These precystic forms are probably formed by division of the larger individuals.

3. **Cyst.**—A cyst wall is secreted round a precystic amœba which has become spherical. The nucleus divides to form two nuclei, these divide to form four, and the four divide again to give the eight nuclei charac-

teristic of the mature cyst (Fig. 101). Occasionally, a further division will take place, giving rise to sixteen nuclei. The cysts with sixteen nuclei, though uncommon, are much more frequently encountered than the eight-nuclear cysts of *E. histolytica.* Very rarely, cysts with a larger number of nuclei occur. During the process of nuclear multiplication some of the nuclei may cease to divide, so that an irregular number of nuclei of unequal size may result. According to Dobell (1919), soon after encystment, a glycogen vacuole forms in the cytoplasm, and this reaches its maximum development at the two-nuclear stage. After this it gradually shrinks till at the eight-nuclear stage it has disappeared. In the writer's experience the precystic amœbæ themselves may possess a large vacuole or a series of vacuoles which run together after encystment has occurred. This vacuole, however, is not always present.

The cysts of *E. coli* vary in diameter from 10 to 30 microns. They usually measure from 15 to 20 microns, but larger ones may occur, as recorded by the writer and O'Connor (1917), who saw one measuring 38 by 34 microns. The commonest type of cyst met with in the stool is one containing a clear cytoplasm in which are embedded the eight nuclei (Fig. 101, 4 and 8).

In most cases there occur also a smaller number of cysts of a different type. These are usually larger than the ones just mentioned, and have a large central glycogen vacuole which reduces the cytoplasm to a thin layer lining the cyst wall (Fig. 101, 10-12). There are usually two nuclei, which generally lie at opposite poles of the cyst. They often appear as if flattened against the cyst wall by pressure of the vacuole. In other cases the vacuole is smaller, and there is a thicker layer of cytoplasm. The vacuole contains glycogen, which stains brown with iodine (Plate II., 2, p. 250). More rarely cysts of this type may be seen with four nuclei, and still more rarely with eight nuclei. A modification is occasionally seen in which a series of vacuoles occurs round the periphery of the cyst (Fig. 101, 14), while the cytoplasm with the two, four, or eight nuclei may occupy its centre. In optical section such cysts have a cartwheel appearance. Dobell (1919) considers that the vacuole occurs in the normal course of development, and reaches its maximum size at the two-nuclear stage, and that it then disappears. It seems to the writer, however, that these two-nuclear cysts with the large vacuole have an abnormal appearance, and it is difficult for him to believe that the eight-nuclear cysts with their particularly clear cytoplasm have been developed from the coarsely vacuolated binucleated cysts, which, moreover, are usually larger than the eight-nuclear cysts present at the same time. On a few occasions the writer has examined material containing precystic amœbæ with perfectly clear cytoplasm, and has seen in the same material cysts

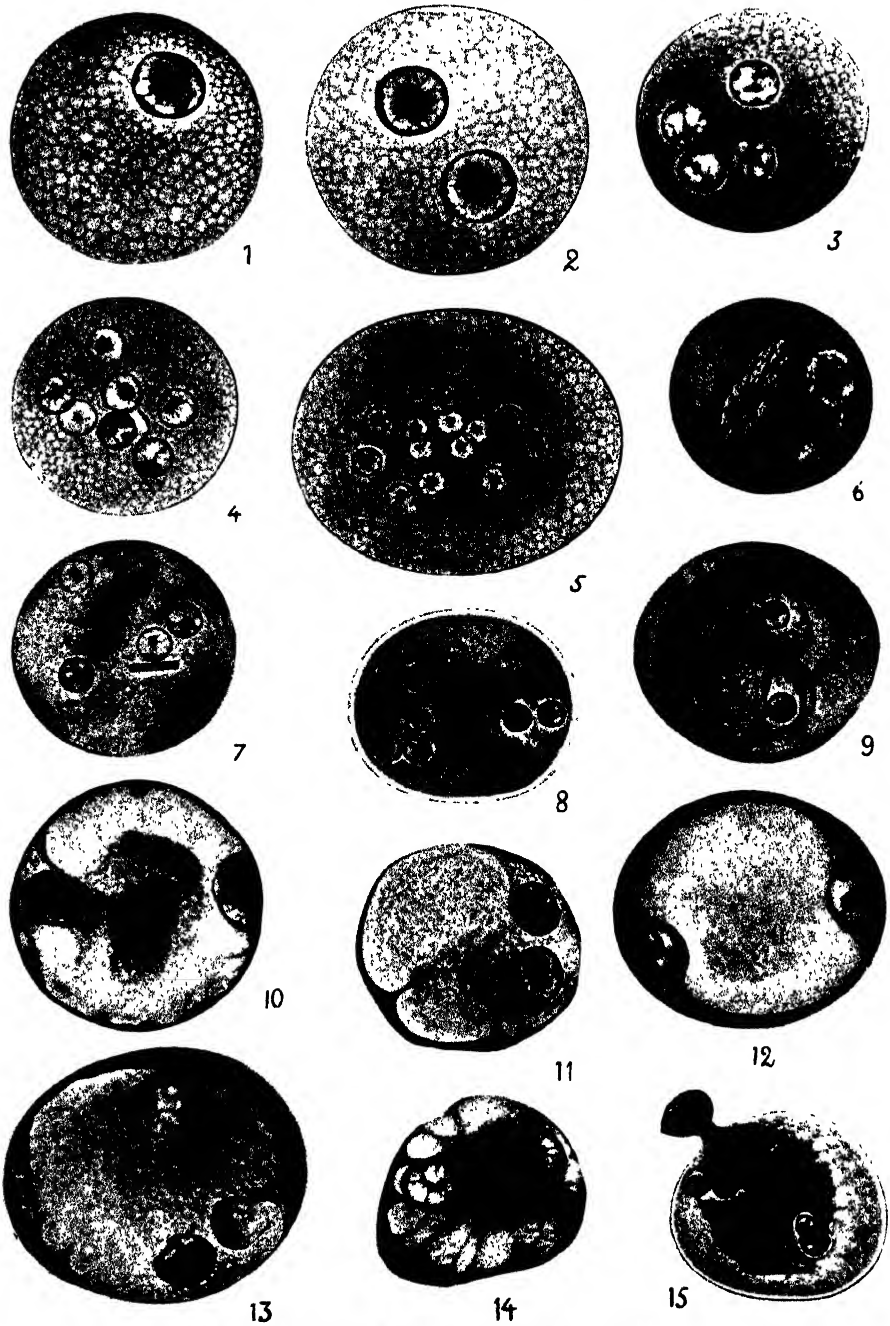


Fig. 101.—*Entamœba coli*: Encysted Forms ($\times$ 2,000). (Original.)

[*For description see opposite page.*

with similar cytoplasm containing one, two, four, and eight nuclei in which no indication of vacuole formation has been evident. He has regarded these as representing the normal encystment process of *E. coli* (Fig. 101, 1-4).

The binucleate cysts with large vacuole appear to be derived from very vacuolated and abnormal-looking precystic amœbæ. It is possible that the real explanation is that in some cases no vacuole is formed at all, in others that one of moderate size occurs and is ultimately absorbed, and that in others again there is an excessively large vacuole formed as an abnormality, and that this prevents the subsequent development of the nuclei. In the case of *E. histolytica* the cysts frequently, though not invariably, contain a vacuole of moderate size which does not impede nuclear division.

It was suggested by the writer and O'Connor (1917) that there probably occur races of *E. coli* in which the average size of the cyst differs, as in *E. histolytica*. Matthews (1919), by measurement of a large number of cysts, demonstrated that this was actually the case.

The nuclei within the cysts have the same structure as that of the adult amœbæ. According to Dobell (1919), the karyosomes are invariably excentric in position, and he believes that it is usually possible to determine with certainty whether the cyst is one of *E. coli* or *E. histolytica* from the nuclear structure alone, provided the cysts have been properly fixed and stained. In the nucleus of *E. histolytica* the karyosome is central.

The arrangement of the nuclei within the cyst is subject to variations. Usually, they are distributed irregularly through the cytoplasm, and careful focussing at different levels is necessary in order to see and count them. At other times they are grouped together, sometimes closely, at the centre of the cyst, where the cytoplasm may be denser than at the periphery.

Chromatoid bodies, first seen by Grassi (1879), and later by Casagrandi and Barbagallo (1897), are occasionally seen in cysts of *E. coli* (Fig. 101, 8). They are usually not so definitely rod-like as those in cysts of *E. histolytica*, and may be in the form of one or more lobulated bodies or numerous, small, irregularly-shaped fragments. Sometimes they are filamentous in form, and the cytoplasm may be traversed by a kind of network of these structures (Fig. 101, 9). The writer has seen cysts in which acicular

1-4. Normal method of encystment, showing one to eight nuclei and absence of vacuole.
5. Large form with sixteen nuclei. **6.** Form with two nuclei in division.
7. Four-nucleated stage with included bacillus.
8. Form with eight nuclei and chromatoid bodies.
9. Form with eight nuclei and filamentous structures.
10-12. Forms with large central glycogenic vacuole and two nuclei.
13. Form with large central glycogenic vacuole and four nuclei.
14. Form with two nuclei and large peripheral vacuoles.
15. Ruptured eight-nucleated stage with hernia-like protrusion.

bodies are arranged at the periphery of the cyst in a tangential manner, while leaving the central cytoplasm, which contains the nuclei, clear. These acicular bodies were similar to certain bacteria which occurred in the stool, and in shape resembled Charcot-Leyden crystals. The possibility of their being parasitic in nature has to be considered.

Schaudinn (1903) described a process of autogamy in the cyst of *E. coli*. The nucleus of the encysted amœba divided into two nuclei, which took up positions at opposite poles of the cyst. Each of these nuclei then gave off chromatin material into the cytoplasm, and then divided to form two pairs of nuclei, one of each pair being a migrating nucleus and the other a stationary one. The migrating nucleus of each pair then passed across the cyst and united with the stationary nucleus of the opposite pair. In this way a two-nuclear stage was again reached. Each nucleus then divided, and the daughter nuclei repeated the division so that a total of eight nuclei resulted. The writer (1907) observed certain changes in the cysts of *E. muris* of mice which seemed capable of a similar interpretation. The observations of Schaudinn have never been confirmed, and it is abundantly evident that no such autogamy process occurs in the development of the cysts of any entamœba. The eight nuclei undoubtedly result from straightforward repeated divisions. Mathis and Mercier (1917) expressed the opinion that the usual type of cyst with eight nuclei were gamete-producing cysts, which in the next host liberated eight amœbæ which conjugated in pairs. The cysts with a larger number of nuclei were regarded as schizogonic cysts, which were presumed to give rise to sixteen daughter amœbæ which grew into adults without conjugation. The figures they give are quite unconvincing, and it is evident from their account that they have not produced sufficient evidence in support of their view.

PATHOGENICITY.—There is no evidence that *E. coli* can be pathogenic to man. That infection is brought about by the ingestion of cysts was demonstrated by Walker and Sellards (1913), who succeeded in infecting seventeen of twenty men on whom experiments were conducted. The infection gave rise to no symptoms, but cysts appeared in the stools in one to eleven days.

The many attempts made by the writer to infect animals with *E. coli* have failed. In conducting such experiments it must be remembered that many animals harbour amœbæ of the *E. coli* type, and that they produce cysts which cannot be distinguished from those of the human amœba. Kessel (1924*a*) reports the successful infection of monkeys with *E. coli*.

Casagrandi and Barbagallo (1897) claimed to have seen the emergence of amœbæ from cysts which had been fed to cats. They supposed that the cyst wall ruptured, and that eight amœbæ escaped from the cyst.

No other observer has been able to repeat this observation, and though it is clear that the cyst must liberate an eight-nucleate amœba or eight uninucleate small amœbæ, this has not been conclusively demonstrated. Kessel (1923*a*) believes that he has succeeded in infecting rats with *E. coli*.

CULTIVATION.—Though several observers claim to have cultivated *E. coli* on the surface of solid agar media in all cases the amœbæ have proved to be coprozoic organisms. Boeck and Drbohlav (1925) succeeded in maintaining *E. coli* for three days in the medium devised for the culture of *E. histolytica*. Drbohlav (1925*d*) and Thomson, J. G. and Robertson (1925) have been more successful and have kept strains growing for two or three months.

ABERRANT FORM OF E. COLI.—Mention must be made of an amœba to which Kofoid and Swezy (1921, 1921*a*) have given the name

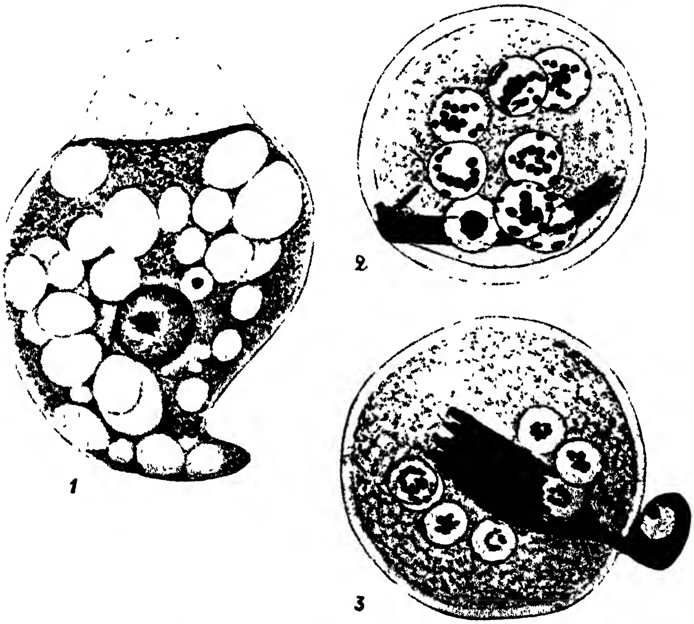


FIG. 102.—FREE AND ENCYSTED FORMS OF "*Councilmania lafleuri*" (× 2,000). (AFTER KOFOID AND SWEZY, 1921, SLIGHTLY REDUCED.)

1. Free form with characteristic nucleus and clear pseudopodium.
2. Encysted form with eight nuclei and chromophile ridge.
3. Cyst producing first bud through the pore.

Councilmania lafleuri (Fig. 102). It is claimed that this is a distinct amœba which has been confused hitherto with *Entamœba coli*. It is supposed to show in its free stage some of the characters of *E. histolytica*, such as activity, development of ectoplasm, formation of clear pseudopodia, ingestion of red blood-corpuscles, and other features which are those of *E. coli*, as distinct nucleus, vacuolation, ingestion of bacteria,

and the production of an eight-nucleated cyst. The nucleus differs from that of *E. coli* in that the karyosome is dispersed instead of being a compact granule. During mitotic division of the nucleus it is claimed that eight chromosomes are present in place of the six which *E. coli* is said to possess. The most characteristic feature, however, and the one on which the new genus is based, is that in the host in which the cysts are formed the encysted amœba buds off, through a pore in the cyst, eight small amœbæ. Associated with the pore is a deeply staining band termed the chromophile ridge. As has been explained above, the exact method of exit of *E. coli* from its cyst is not known, so that the budding process cannot be held to distinguish the new genus *Councilmania* from *Entamœba.* There would be more reason to place *Entamœba gingivalis* in another genus, because encysted forms have never been discovered. It appears, however, from the description and figures, that the supposed budding process through a pore is most reasonably explained as a result of rupture of the cyst and the consequent extrusion by pressure or collapse of the cyst, of hernia-like portions of the cytoplasm together with the nuclei. The writer has seen exactly comparable appearances in cysts of *E. coli* which have been ruptured by pressure of the cover-glass. As the liquid beneath the cover-glass evaporates the pressure on the cysts is increased, so that rupture takes place and portions of cytoplasm with nuclei can be seen to escape. Similar ruptured cysts are often encountered in the ordinary stained preparations made by the smear method. The writer has preparations containing ruptured cysts which may be in the two, four, or eight nucleated stage (Fig. 101, 15). They contain chromatoid bodies, and bear a striking resemblance to the budding cysts described by Kofoid and Swezy (1921*a*). Werner (1912) gave a figure of a similarly ruptured cyst showing a hernial protrusion including two nuclei. Casagrandi and Barbagallo (1897) figure a cyst which is supposed to illustrate the natural emergence of amœbæ, but it is not improbable that they were observing an artificially ruptured cyst. The writer has seen in stained preparations similar ruptured cysts of *E. histolytica* with bud-like extrusions containing one of the four nuclei. In view of the work of Sellards and Theiler (1924), who have shown that kittens may be infected with *E. histolytica* by injecting material containing cysts only, it is possible that *E. coli* may sometimes emerge from its cyst while in the large intestine, and that some of the appearances of budding may be due to this. It is nevertheless a fact that artificial rupture of cysts of *E. coli* will give rise to forms which are said to be characteristic of *C. lafleuri*. The deeply staining band called the chromophile ridge, which is supposed to have some connection with the development of the pore, is probably an artifact in many cases, the result of irregularities in staining produced by folds or

creases in the cyst wall, or disturbance of the cytoplasm. In some cysts the structures called chromophile ridges are undoubtedly chromatoid bodies. Such statements as " chromatoidal body exhausted in the formation of chromophile ridge," made in connection with the cyst reproduced at Fig. 102, *2*, are quite incomprehensible. It is probable that Kofoid and Swezy were dealing in some cases with mixed infections of *E. coli* and *E. histolytica*. The writer (1922*a*, 1925) stated his reasons for regarding the name *Councilmania lafleuri* as a synonym of *Entamœba coli*. In a later communication Kofoid, Swezy, and Kessel (1924) reaffirm their belief in *Councilmania lafleuri*, and bring forward a number of further observations which they consider establish the validity of the species. After carefully reading their paper, the writer still believes that there is no justification for the genus *Councilmania*, and that the characters which distinguish *C. lafleuri* from *E. coli* fall within the range of variation of *E. coli* itself. Gunn (1922) has examined some of the cases from which Kofoid and Swezy described *C. lafleuri*. He has found that the amœbæ present were actually *E. coli*.

Having discovered similar " budding cysts " in rats and mice, *E. muris* and *E. decumani* are placed in the genus *Councilmania* as *C. muris* and *C. decumani* by Kofoid, Swezy and Kessel (1923), while it is also claimed by Kessel (1923*a*) that rats and mice can be infected with *C. lafleuri*, and that the amœba retains its characters in these animals. Apart from the " budding " through a pore in the cyst, which the writer believes is a rupture, the main points which, it is claimed, distinguish the genera *Entamœba* and *Councilmania* are the character of the cytoplasm and its inclusions, the clear pseudopodia, the type of movement, and finally the dispersed karyosome. There is very great difficulty associated with the identification and counting of chromosomes in nuclei of the type possessed by these amœbæ, so that the chromosome number quoted for *C. lafleuri*, *C. muris*, *C. decumani*, *E. coli*, and *E. histolytica* (8, 6, 4, 6, 6) cannot be accepted as finally established. Kofoid and Swezy (1921*a*) state that they have encountered *E. muris* in man. *E. muris* of rats and mice so closely resembles *E. coli* of man that the writer is at a loss to know how they arrived at their diagnosis, especially as Kofoid, Swezy and Kessel (1923) adopt the view that the amœba belongs to the genus *Councilmania*.

Entamœba gingivalis (Gros, 1849) Brumpt, 1910.—This amœba, which is parasitic in the human mouth, was first seen by Gros (1849) in Russia. He gave it the name *Amœba gingivalis*, which was emended by Brumpt (1910*a*) to *Entamœba gingivalis*. The organism was seen by Steinberg (1862), who gave it the name *Amiba buccalis*, and by Grassi (1879), who named it *Amœba dentalis*. Doflein (1901) referred to it as *Amœba kartulisi*, and Kartulis (1906) as *Entamœba maxillaris*. It has been described

under various names by different observers from the material obtained from carious teeth or abscesses in the oral and pharyngeal regions. Smith and Barrett (1915), and in the same year Bass and Johns (1915), studied this amœba, and concluded that it was probably the cause of pyorrhœa alveolaris, and that it invaded the tissues like *E. histolytica.* There is no conclusive proof that *E. gingivalis* is pathogenic in any way or actually invades the tissues, so that it is safer to regard it as a saprophitic organism which lives in the mouth, especially in any pockets which may form in suppurative conditions, along with the numerous spirochætes, bacteria, and trichomonas. The observation of Lynch (1915*b*) that *E. gingivalis* may occur in material obtained from the interstices of sets of false teeth worn by individuals with no natural teeth at all and perfectly healthy gums seems difficult to reconcile with the view that the amœba is, like *E. histolytica*, a tissue parasite. More recently, under the name of *E. macrohyalina*, Tibaldi (1920) has described an amœba obtained from the tonsil. This again is probablv no other than *E. gingivalis*, which has been shown by Smith, Middleton, and Barrett (1914) to invade the crypts of the tonsil under suitable conditions, just as trichomonas and the other organisms of the mouth may do. The bodies which Artault (1898) discovered in a cavity of the lung, and which he named *Amœba pulmonalis*, are probably the same as those referred to as *Entamœba pulmonalis* by Brumpt (1913*c*). If they are amœbæ, which is by no means clear, they may be identical with the oral form. It has been suggested that *E. histolytica* may invade the mouth, and that *E. gingivalis* is in reality that species. There seems to be no ground whatever for this conclusion, nor is there any reason to suppose that more than one species of amœba inhabits the mouth. The many names that have been given are the result of observations on degenerate amœbæ, just as has occurred in the case of *E. histolytica* and *E. coli.* Petzetakis (1923 and 1923*b*) claims to have observed *E. histolytica* in material coughed up from the lungs in a type of broncho-pneumonia (see p. 193).

E. gingivalis is a fairly active amœba when observed on the warm stage, and possesses an ectoplasm which is even clearer than that of *E. histolytica.* Kofoid and Swezy (1924*a*) note that sometimes in apparently normal amœbæ there is no distinction between ectoplasm and endoplasm. They state that a definite superficial pellicle is always present. As regards its activity, *E. gingivalis* is perhaps intermediate between *E. histolytica* and *E. coli*, and there is a greater tendency to the formation of several pseudopodia at one time. These are smaller in comparison with the size of the amœba than are those of *E. coli* and *E. histolytica*, and according to Jepps (1923*a*), who has studied the organism in Malaya, they are never formed in the eruptive manner so characteristic

of those of *E. histolytica*. They are clear, and appear to consist of ectoplasm alone when this layer is sharply defined. Jepps, as well as Kofoid and Swezy (1924*a*), note that during progression the amœba may become elongated, while the hindermost portion becomes drawn out into a tail-like process to which adhere collections of bacteria, leucocytes, and débris. The amœbæ vary in diameter from 10 microns upwards, but they are rarely seen with a diameter above 20 microns (Fig. 103). Forms up to 40 microns in diameter have, however, been described. There is distinguishable a clear, narrow ectoplasmic layer and a highly vacuolated granulated endoplasm. The many food vacuoles contain a variety of structures, some of which stain black with iron hæmatoxylin, and are

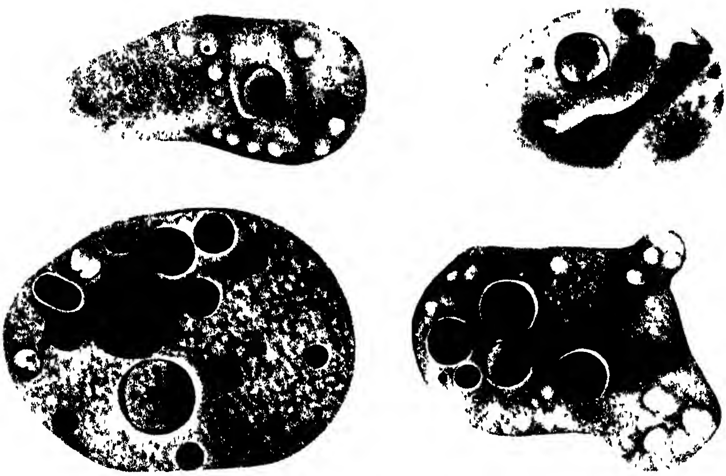


FIG. 103.—*Entamœba gingivalis* FROM SCRAPINGS FROM A CARIOUS TOOTH (× 2,000). (ORIGINAL.)

In three of the amœbæ are seen the large ingested bodies of doubtful nature.

probably the nuclei of degenerate pus or tissue cells. Smith and Barrett (1915) and others state that red cells are sometimes ingested, but the majority of observers, including the writer, have obtained no evidence of this. The food vacuoles also contain bacteria of various kinds. The nucleus is distinctly smaller than that of *E. coli*, which it resembles, however, in general features. The nuclear membrane is generally lined with closely packed granules of chromatin, which in stained specimens appear as a uniform black ring. There is a karyosome which may be surrounded by a clear area, as in the nuclei of *E. coli* and *E. histolytica*. According to Dobell (1919), the karyosome is either central or excentric in position, and there is no chromatin upon the linin network. As in the case of the nuclei of *E. coli* and *E. histolytica*, some observers have maintained that there is a centriole within the karyosome.

Kofoid and Swezy (1924*a*) maintain that all descriptions of the nucleus hitherto given are inaccurate, and that definite differences not previously noted distinguish it from the nucleus of *E. histolytica*. They state that the karyosome is not always a single granule, as in *E. histolytica*, but is often composed of a group of granules, and that the halo round the karyosome is granular and large, in contrast with the clear and relatively smaller halo of *E. histolytica*; furthermore, the intermediate zone between the nuclear membrane and halo is clear in *E. gingivalis* and granular in *E. histolytica*, while in the former the chromatin is less regularly arranged and more liable to clumping on the nuclear membrane. Whether such minute differences are sufficiently constant to justify the determination of species future investigations alone will show.

Reproduction of *E. gingivalis* probably takes place by binary fission, and the binucleate forms occasionally seen must represent a stage in this process. The division has never been followed in detail.

Craig (1916) has recorded the finding of cysts, but it is evident from his figures that the structures described were not cysts at all. Similarly, Smith and Barrett (1915), and Nowlin (1917), described as cysts structures which were more than doubtful. The writer has examined *E. gingivalis* on many occasions, but was never able to discover encysted forms. This has been the experience of Dobell (1919), Kofoid and Swezy (1924*a*), and other workers. It is probable that cysts occur, but if they have ever been seen, no convincing description has yet been given.

E. gingivalis can easily be studied in material obtained from carious teeth or in pus squeezed from pyorrhœal pockets. In the writer's experience the amœbæ sometimes appear to be absent in particularly foul mouths when they might be expected to be present, while on other occasions they have been found in the mouths of people who are very particular as to their dental toilet.

Attempts to infect animals with *E. gingivalis* have not been successful. Goodrich and Moseley (1916) have noted that an organism indistinguishable from *E. gingivalis* may be found in pyorrhœic conditions in dogs, while Nieschulz (1924*c*) has described as *E. gingivalis* var. *equi* a similar form from the accumulations round the teeth of horses.

Tibaldi (1920) has recorded the discovery of *E. gingivalis* in the human tonsil. He has also described as *E. macrohyalina* an amœba of another type which he has found in two cases of tonsillitis. This amœba is considerably larger than *E. gingivalis*, and may reach a diameter of 40 microns. It has, moreover, a well-marked ectoplasm and a different type of nucleus, though it must be admitted the figures given suggest a faulty fixation. It is possible, as noted by the writer (1922*a*), that *E. gingivalis*, which usually lives as a saprophyte, may become modified

in appearance when it inhabits an inflamed tonsil. This is probably the explanation of the curious amœbæ which have been described from abscesses in the jaw and mouth. Though Tibaldi has drawn attention to an amœba which differs from the usual form of *E. gingivalis*, he has not produced any evidence, apart from its size, to justify its separation as a distinct species.

Drbohlav (1925*c*), Howitt (1925) and Dobell (1926) have cultivated *E. gingivalis*. Drbohlav failed to infect kittens with the cultured forms (see p. 1297).

ENTAMŒBÆ OF MONKEYS.

Musgrave and Clegg (1904) stated that they had occasionally observed natural amœbic infections of monkeys in the Philippines, and the writer

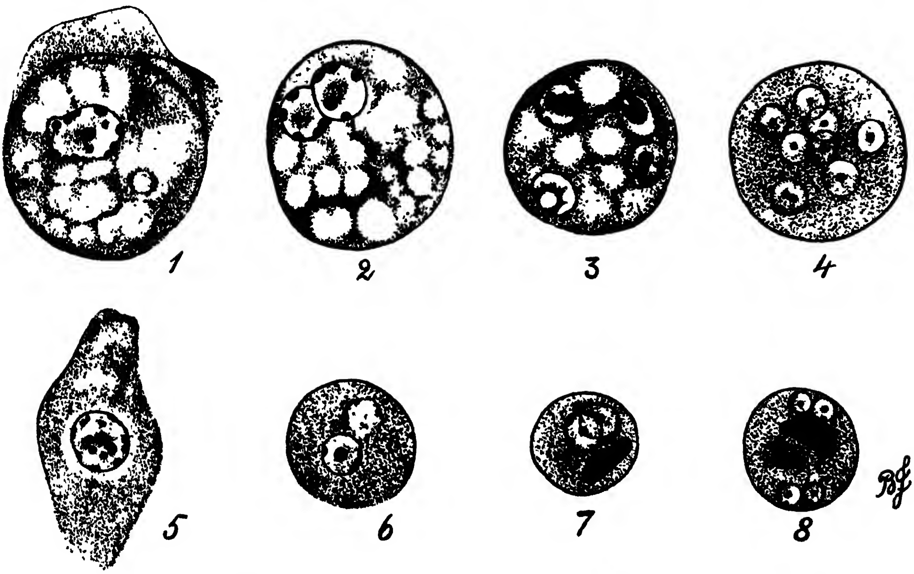


FIG. 104.—ENTAMŒBÆ FROM THE INTESTINE OF MONKEYS (× *ca.* 1,300). (AFTER MATHIS, 1915.)

1. Free form of *E. pitheci*. 2-4. Encysted forms of *E. pitheci*.
5. Free form of *E. nuttalli*. 6-8. Encysted form of *E. nuttalli*.

(1909) observed cysts which were indistinguishable from those of *E. coli* in a monkey in Khartoum. Brumpt (1909*a*) observed similar cysts and free amœbæ in *Macacus sinicus*, while Noc (1909) observed cysts 10 to 12 microns in diameter in three monkeys in Saigon. Castellani (1908) observed an amœbic abscess of the liver in a *Macacus pileatus* in Colombo, and proposed the name *Entamœba nuttalli* for the amœba. Mathis (1913) published an account of an amœba observed by him in *Macacus rhesus* and *Macacus tcheliensis* of Tonkin. He found two distinct types, one resembling *E. coli* of man in that it produced eight-nucleated cysts (Fig. 104,

1-4), and the other like *E. histolytica*, with cysts containing four nuclei and chromatoid bodies (Fig. 104, 5-8). Employing the generic name *Löschia* proposed for the entamœbæ of man by Chatton and Lalung-Bonnaire (1912), he named these forms *L. legeri* and *L. duboscqi* respectively. Prowazek (1912*a*), however, had previously described and named *E. pitheci*, a form which he had seen in an orang-outang and which resembled *E. coli*, though, according to Dobell (1919), he was probably dealing with more than one species. Swellengrebel (1914) gave the name *E. chattoni* to an amœba seen by him in *Macacus rhesus*. It was of the *E. histolytica* type. Behrend (1914) observed cysts in the fæces of a *Macacus rhesus*. They varied in diameter from 8 to 25 microns, some having four and others eight nuclei. Macfie (1915*a*) also saw amœbæ in a monkey (*Cercopithecus petaurista*) of West Africa. It was associated with dysentery, of which Macfie judged it to be the cause. He named it *Entamœba cercopitheci*. Eichhorn and Gallagher (1916) recorded an outbreak of amœbic dysentery amongst spider monkeys (*Ateles ater*) in America. The amœba is referred to as *Amœba ateles* by these authors and as *Entamœba ateles* by Suldey (1924).

McCarrison (1919) stated that monkeys employed by him in nutrition experiments in India were very liable to attacks of amœbic dysentery. Bach (1923) described the cysts and free forms of an amœba of the *E. histolytica* type which he discovered in a *Macacus rhesus* which had been in captivity in Germany for sixteen years. Suldey (1924) has described a case of spontaneous amœbic dysentery in the chimpanzee. The amœba had all the characters of *E. histolytica*. Amœbæ of this type have been seen by Kessel (1924*a*) in monkeys in China.

Most of the observations on the amœbæ of monkeys have been casual ones, so that the descriptions given do not necessarily represent the normal appearance of the healthy amœbæ. It is evident that monkeys may harbour two forms—one, *E. pitheci* Prowazek, 1912 (=*E. legeri* Mathis and Mercier, 1917), which resembles *E. coli*; and the other, *E. nuttalli* Castellani, 1908 (=*Löschia duboscqi* Mathis, 1913=*E. chattoni* Swellengrebel, 1914=*E. cercopitheci* Macfie, 1918=*E. ateles* Suldey, 1924), which resembles *E. histolytica*. The latter is liable to produce amœbic dysentery and abscess of the liver. It is open to question if these forms are really distinct from *E. coli* and *E. histolytica*.

Mello (1923) in Italy has found that species of *Macacus* harbour either *E. pitheci* or *E. nuttalli*. The latter is often associated with dysentery, and the injection of its cysts *per rectum* produced dysentery in three kittens, which passed large numbers of amœbæ. In a young orang-outang an amœba of another type is described. It measured 25 to 35 microns, and its mature cyst had eight nuclei. It differed from *E. pitheci* chiefly in

the fact that cysts and free forms with over twenty nuclei occurred. The multinucleated free forms are regarded as schizonts, and a figure shows what the author regards as division into daughter amœbæ. It is far from clear that these free forms are not cysts, and the figure illustrating the escape of the daughter amœbæ from an enclosing membrane which he says is present might well be interpreted as a ruptured cyst from which the nuclei are being extruded by pressure. Though the author refers to the amœba as a new species, *E. multinucleata*, it is evident that he may have been dealing with multinucleated forms of *E. pitheci*, and that this amœba comes into line with *E. coli*, in which similar stages are by no means uncommon. In stained and cleared preparations, as pointed out above, it is often exceedingly difficult to decide whether a form is actually encysted or not. The writer has seen free forms and cysts of an amœba resembling *E. pitheci* in *Cercopithecus* sp. of West Africa, and with Dr. G. C. Low the cysts alone in the fæces of a gorilla.

Dobell (1926) has cultivated from monkeys four species of amœba including *E. nuttalli*. With the last, the complete history of which, including excystation, has been studied in cultures, he has produced in kittens a dysentery which differs in certain respects from that resulting from inoculation of *E. histolytica*.

Brug (1923) has discovered in *Macacus cynomolgus* a small race of an amœba corresponding with the small race of *E. histolytica* in man. On the assumption that these human amœbæ represent a distinct species (*E. tenuis*), he gives the name *E. cynomolgi* to the form in the monkey. It is possible, however, that it is merely a small race of *E. nuttalli*.

ENTAMŒBÆ OF OTHER ANIMALS.

Entamœbæ are of common occurrence in the intestine of animals, while occasionally they occur in the mouth of the dog and horse, as noted above (p. 224). Spontaneous amœbic dysentery in dogs has been described by Kartulis (1891, 1913) in Egypt, Darling (1915) in Panama, and Ware (1916) in India, Fischer (1918) in China, and Bauche and Motais (1920) in Cochin-China. In one case noted by Kartulis the dysentery was associated with abscess of the liver. As the dog is known to be infectible with *E. histolytica*, it seems probable that it was actually this species which was producing the disease. Darling (1915), without differentiating it from *E. histolytica*, proposed the name *E. venaticum* for the amœba producing canine amœbic dysentery. Franchini, F. (1920–1923), recorded a case of spontaneous amœboid dysentery in a cat in Italy. It was concluded that the amœba was *E. histolytica*.

Rats and mice are very commonly infected with amœbæ [*E. muris* (Grassi, 1879)] which closely resemble *E. coli* both in the free and encysted stages (Fig. 105). Brug (1919*a*) has noted that while *E. muris*, regarded by Rudovsky (1921) as a variety *E. muris-decumani*, is the common

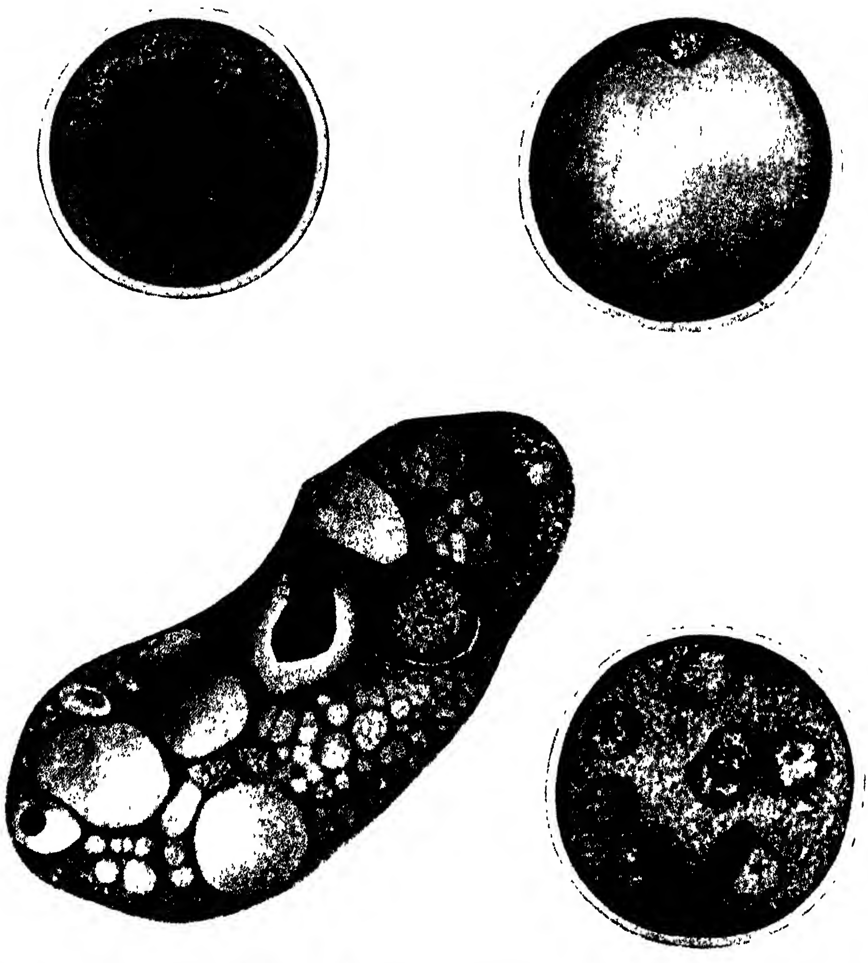


FIG. 105.—*Entamœba muris* FROM THE INTESTINE OF A WHITE MOUSE (×3,000). (ORIGINAL.)

One unencysted form and three encysted forms with two, four, and eight nuclei.

form seen in rats, occasionally amœbæ of the *E. histolytica* type are met with. Lynch (1915) and Chiang (1925), who also found amœbæ of this type in rats, believe that rats may actually act as carriers of *E. histolytica* (see p. 206).

Kofoid, Swezy and Kessel (1923), in their conception of a genus *Councilmania,* have stated that the common amœbæ of rats and mice belong to this genus, in that they possess clear pseudopodia and dispersed karyosomes, and are able in the encysted stage to form buds through a pore, associated with which there may be a problematic structure called the chromophile ridge. They accordingly transfer *E. muris decumani* and *E. muris* to the genus *Councilmania* as *C. decumani* and *C. muris.* Furthermore, Kessel (1924) states that in the rat there occurs an amœba which differs from *C. decumani* in that it forms granular pseudopodia, has a compact karyosome, and does not form buds, in which respects it resembles *E. coli.* It is given the name *E. ratti.* As explained by the writer (1922*a*, 1925) there are no grounds for retaining the genus *Councilmania* (see p. 219), so that if Kessel's claims regarding these amœbæ are correct there are to be recognized in rats *E. decumani* and *E. ratti*, and in mice *E. muris.* According to him, *E. decumani* and *E. muris* may be transferred to both rats and mice. The writer (1925) feels convinced that much more work will have to be done before the claims regarding these three species can be accepted. It is possible that the amœba of the rat differs from that of the mouse, but at present it seems safer to regard the amœbæ of both rats and mice as *E. muris.*

An amœba with free and encysted stages of the *E. coli* type was discovered by Brug (1918 *a*) in rabbits. He named the organism *E. cuniculi.* Rudovsky (1923) saw an *Entamœba* in hares. A similar form in guinea-pigs was named *E. cobayæ* by Walker (1908), and was again referred to by Chatton (1918*c*) as *E. caviæ.* It has been seen by the writer in guinea-pigs on several occasions, while the free and encysted forms of an amœba resembling *E. muris* were met with in the jerboa in the Sudan. Leger, M. (1918), has also recorded an amœba of guinea-pigs. He noted that encysted forms of the four-nuclear type were present, while Holmes (1923) has observed cysts like those of *E. coli.*

Theobald Smith (1910) discovered amœbæ in sections of intestinal ulcers in the large intestine of pigs in America. He did not consider them as pathogenic, but believed they had invaded ulcers which were due to some other cause. They varied in diameter from 8 to 10 microns, and each had a single nucleus with a small central karyosome. Prowazek (1912) described as *E. polecki* an amœba found by him in pigs in Saipan. He claimed to have seen the same amœba in a child also, but this was probably a precystic form of *E. histolytica.* Prowazek's pig amœba varied in diameter from 10 to 12 microns, and had a single nucleus which, in some of his figures, is evidently of the entamœba type. Hartmann (1913), after examining some of Smith's sections, proposed to name the amœba *E. suis,* though admitting its possible identity with

E. polecki. Nöller (1921) states that Feibel in Germany had seen this amœba in pigs, while Cauchemez (1922 *a*) describes it from pigs in France. The writer has seen it in pigs in England, and it was also met with by O'Connor in the Ellice Islands. According to Cauchemez, the amœba is nearly always uninucleated, and varies in diameter from 5 to 12 microns when round. When elongate, it measures 15 by 5 microns. Rarely binucleate forms were seen. The amœbæ resemble the precystic forms of *E. histolytica*. Nöller (1922), who emphasizes its resemblance to *E. histolytica*, states that the amœba varies in diameter from 12 to 25

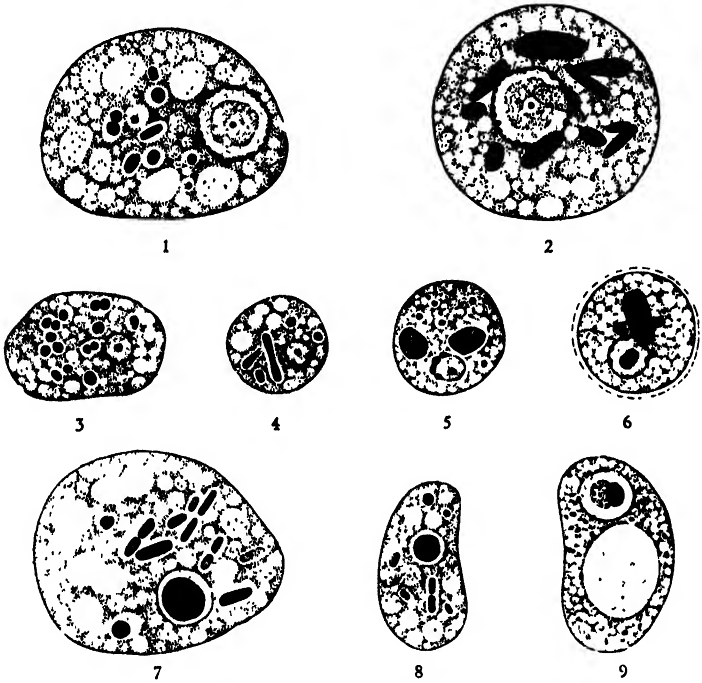


FIG. 106.—INTESTINAL AMŒBÆ OF PIGS (×2,500). (AFTER NIESCHULZ, 1923.)
1-2. *Entamœba polecki*, free forms. 3-5. *E. debliecki*, free forms.
6. *E. debliecki*, encysted form. 7-8. *Iodamœba bütschlii*, free forms.
9. *I. bütschlii*, encysted form.

microns, and that uninucleate cysts 12 to 15 microns in diameter occur. The latter may contain numerous splinter-like chromatoid bodies. Douwes (1921) described four-nucleated cysts with a diameter of 5 to 8 microns. Whether these are the mature cysts of a small race of the entamœba of the pig or some other form is not clear. The correct name for this amœba is evidently *E. polecki* Prowazek, 1912, though some of the forms figured by Prowazek undoubtedly do not belong to this amœba. The name *E. suis* becomes a synonym. The amœba cultivated from pig

fæces by Walker (1908), and named by him *Amœba intestinalis*, is not a parasitic form at all, but a coprozoic organism.

Nieschulz (1923*a*, 1924*b*) found that pigs harbour two species of *Entamœba*. There is the large form referred to above, and a smaller one not more than 5 to 9 microns in diameter, which he proposes to name *E. debliecki*. Uninucleate cysts with chromatoid bodies are described (Fig. 106.)

Liebetanz (1905) described *E. bovis* from the stomach of cattle. It was redescribed by him (1910) and by Braune (1913), and was said to be 20 microns in diameter. Nieschulz (1922*b*) has met with a smaller, though possibly the same form in cattle in Germany. The amœbæ, which varied from 5 to 10 microns in diameter, had nuclei of the entamœba type. He also saw uninucleated cysts 5 to 12 microns in diameter in the fæces. He was unable to determine with certainty that these cysts were derived from the amœbæ in the rumen.

Fantham (1920, 1921) refers to an amœba called by him *E. intestinalis* (*Amœba intestinalis* Gedoelst, 1911), which occurs in the colon and cæcum of horses in South Africa. No details of the structure are given. He states (1921) that in the fæces he has seen another form which he names *E. equi*. It may contain red blood-corpuscles, and when round has a diameter of 28 to 35 microns. Four-nucleated cysts containing chromatoid bodies and measuring 15 to 20 microns in diameter are also mentioned. It is assumed that it is a pathogenic species.

Swellengrebel (1914) discovered free amœbæ and uninucleated cysts in the intestine of sheep, and proposed the name *E. ovis*. The writer has seen eight-nucleated cysts of the *E. coli* type in goats' fæces. Fantham (1923) gave the name *E. capræ* to an amœba of the goat. Very little is known about these forms. Nieschulz (1923*b*) has found in goats an amœba which appears to be identical with the small *E. debliecki* of pigs.

Fantham (1910*b*) described as *E. lagopodis* an amœba found by him in the intestine of the grouse, *Lagopus scoticus*. Cysts with four nuclei were noted. According to Hartmann (1913), Kuczynski saw a similar form in fowls, but the encysted stages had eight nuclei. Tyzzer (1920) saw the same amœba in American fowls. He noted that in the free and encysted stages it closely resembled *E. coli*. To a form in the duck in S. Africa Fantham (1924) gave the name *E. anatis*. Cysts with one or four nuclei are described.

Frogs harbour amœbæ (*E. ranarum* Grassi, 1879) which Dobell has shown to resemble *E. histolytica* very closely in the free and encysted stages (Fig. 107). So similar were these forms that Dobell (1918) attempted to infect tadpoles by causing them to ingest cysts of *E. histolytica*. The cysts showed no signs of hatching in the intestines of the tadpoles, and were passed unaltered in the fæces. *E. ranarum* was

studied in tadpoles by Collin (1913), who found that the free amœbæ sometimes had as many as thirty nuclei (Fig. 107, 7). He regarded these as schizonts. The amœba was again studied by Mercier and Mathis (1918), who described two types of cysts. The usual form had four nuclei, like the cysts of *E. histolytica*, while the other had as many as sixteen nuclei (Fig. 107, 8). As in the case of *E. coli*, it was conjectured that the

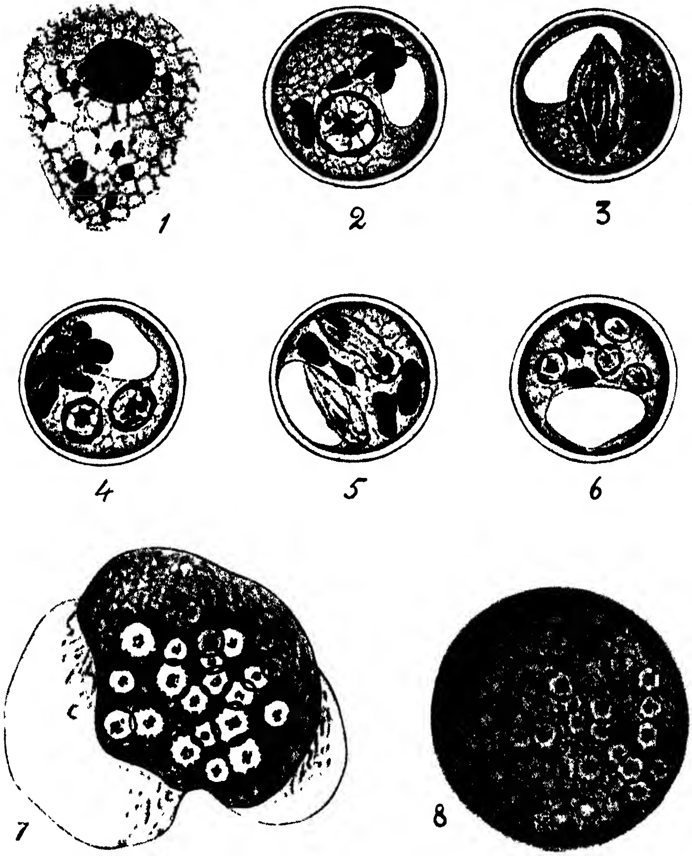


FIG. 107.—*Entamœba ranarum* FROM THE INTESTINE OF THE FROG. (1-6 AFTER DOBELL, 1909; 7 AFTER COLLIN, 1913; 8 AFTER MERCIER AND MATHIS, 1918.)

1. Free form (× 2,000). 2-6. Encysted forms (× 2,000).
7. Multinucleated free form (× 1,000). 8. Multinucleated encysted form (× 1,400).

cysts with a small number of nuclei were gamete-producing cysts, while those with a larger number represented schizogony cysts. No proof in support of this view was obtained (see p. 218).

Ilowaisky (1922) has described spontaneous amœbic abscess of the liver in frogs. The amœbæ present resembled *E. ranarum*, which occurred in the intestine of the same animals. The amœba seen by Chatton

(1910*c*) in the rectum of the newt, *Triton palmatus*, and by Alexeieff (1912) in *Triton tæniatus*, is very possibly *E. ranarum*.

Hartmann (1910*b*) described as *E. testudinis* an amœba of the tortoise, *Testudo græca*. It was also seen by Alexeieff (1912*c*) in *Nicoria trijuga*, a tortoise of Ceylon, while the writer has met with it in *Testudo argentina* and *T. calcarata*. An *Entamœba* of the turtle, *Chelydra serpentina*, of America, which was cultivated by Barret and Smith (1923), has been named *Entamœba barreti* by Hegner and Taliaferro (1924) (see p. 207).

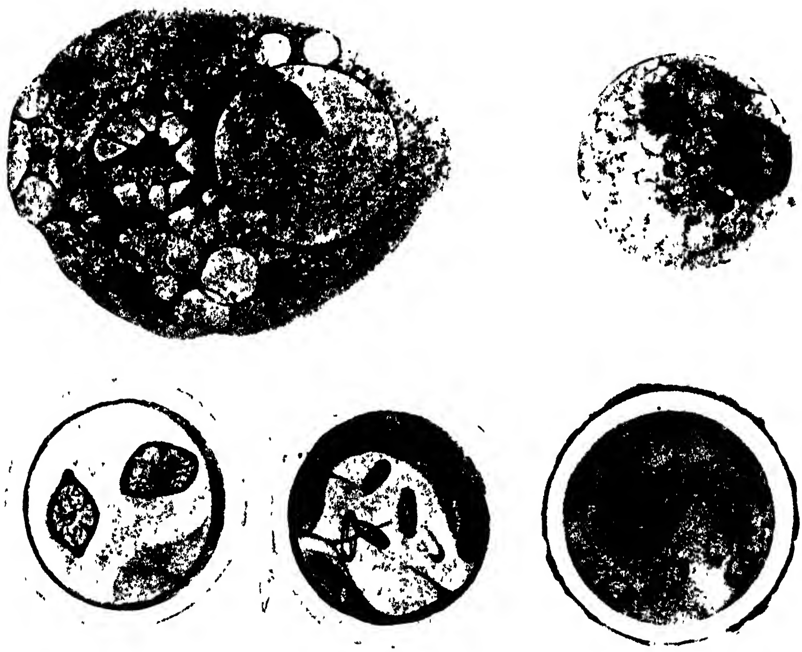


FIG. 108.—*Entamœba minchini* OF TIPULID LARVÆ: FREE AND ENCYSTED FORMS (×3,200). (AFTER MACKINNON, 1914.)

Dobell (1914*a*) gave a figure of an entamœba seen by him in the wall lizard, *Lacerta muralis*. The writer (1921) encountered a similar amœba in Egyptian lizards (*Lacerta agilis* and *Agama stellio*). The free forms were very like those of *E. coli*, while eight-nuclear cysts occurred which were indistinguishable from those of the human parasite. What is probably the same amœba was seen in *Lacerta ocellata* by Franchini (1921*a*). Cunha and Fonseca (1917) described as *E. serpentis* an amœba seen by them in the snake *Drimobius bifossatus* of S. America.

Léger and Duboscq (1904) observed an amœba in the intestine of the marine fish *Box boops* and *B. salpa*. It was studied by Alexeieff (1912), who placed it in a new genus as *Proctamœba salpæ*. According to him,

four-nucleated cysts with chromatoid bodies are produced. It clearly belongs to the genus *Entamœba*.

Amœbæ belonging to this genus occur also in invertebrates. Nöller (1912*a*) discovered a form resembling *E. histolytica* in the vagina of the horse leech. The free forms varied from 4 to 35 microns in diameter, and the cysts, which measure 7 to 11 microns, had four nuclei and chromatoid bodies. Nöller gave the name of *E. aulastomi* to this amœba, which has been cultivated by Drbohlav (1925*e*). Another invertebrate form is *E. minchini*, described from the larvæ of Tipulids by Mackinnon (1914). The free amœbæ were 5 to 30 microns in diameter, while the encysted forms contained a maximum of ten nuclei (Fig. 108).

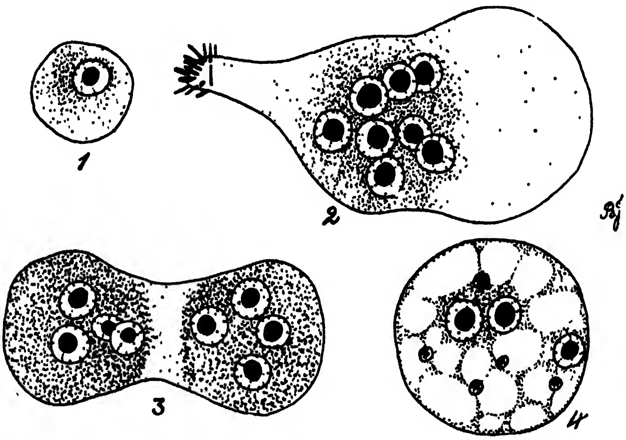


FIG. 109.—*Entamœba mesnili* FROM THE INTESTINE OF LARVÆ OF *Trichocera hiemalis* AND *T. annulata* (× 2,000). (AFTER KEILIN, 1917.)

1. Uninucleated form.
2. Multinucleated form with trailing pseudopodium, to which are adherent bacteria and other débris.
3. Multinucleated form in division.
4. Encysted form with three nuclei.

Keilin (1917) described as *E. mesnili* an amœba which lives in the intestine of the larvæ of the Diptera *Trichocera hiemalis* and *T. annulata*. The amœbæ vary in length from 6 to 24 microns, and in breadth from 4 to 8 microns (Fig. 109). There is a clear ectoplasm and a finely granular endoplasm which is free from food vacuoles. A curious feature of the amœba is that many forms are multinucleate and contain from four to fourteen nuclei according to their size. These multinucleate amœbæ divide to give rise to daughter forms, which are also multinucleate. Sometimes uninucleate forms are budded off, and these apparently increase in size and become multinucleate. Encysted forms measuring 8 to 11 microns in diameter are found. They have two to four nuclei. The nucleus of this amœba contains a large central karyosome, and in this respect differs from the typical nucleus of *Entamœba*, so that it is possible that the amœba belongs to another genus.

Brug (1922) describes as *E. belostomæ* a large amœba from the intestine of the water bug *Belostoma* sp. of Java. The amœba was said to be of the *E. histolytica* type. Cysts, however, were not seen.

Fantham and Porter (1911) saw an amœba which they named *E. apis* in the bee, *Apis mellifica.* It resembled *E. coli.*

Genus **Endamœba** Leidy, 1879.

This genus was created by Leidy (1879) for an amœba of the cockroach (*Periplaneta orientalis*). It was named *Amœba blattæ* by Bütschli (1878), while Leidy gave it the name *Endamœba blattæ.* If it should be proved that this amœba of the cockroach belongs to the same genus as the human forms to which Casagrandi and Barbagallo (1895) gave the name *Entamœba coli,* then the correct generic name for the human and other forms will be *Endamœba,* as many American writers maintain. Cockroaches, however, harbour at least two amœbæ, one of which undoubtedly belongs to the genus *Entamœba.* The other is *Endamœba blattæ,* which, according to Mercier, has such a characteristic nucleus that a distinct genus is justified. Thomson and Lucas (1926) have recently redescribed the amœba. Their description conforms entirely with Mercier's account of the morphology of *E. blattæ.*

Endamœba blattæ (Bütschli, 1878).—This amœba was studied by Schubotz (1905), Janicki (1908, 1909), and later by Mercier (1910), who has given the most detailed account of its structure and life-history. *E. blattæ* lives in the intestine amongst the various nematodes, vegetable and other protozoal organisms which are found there. It varies very much in size, ranging from 10 to 120 microns in diameter (Fig. 110). The average-sized forms measure about 50 microns. The general appearance varies considerably with the quantity and nature of the food inclusions with which the endoplasm may be packed. There is no marked distinction between ectoplasm and endoplasm, but the cytoplasm, which is highly vacuolated, contains smaller vacuoles near the surface than at the centre. The movements are sluggish, and one or two blunt pseudopodia are formed at a time. Sometimes the cytoplasm streams internally in a peculiar manner, which gives the amœba a striated appearance. There is no contractile vacuole.

The nucleus, which differs in many respects from the typical nuclei of members of the genus *Entamœba,* is an ovoid structure measuring 10 to 15 or even 20 microns in its longest diameter. It is limited by a remarkably thick nuclear membrane, within which, even in the living amœbæ, can be distinguished two zones—a peripheral one consisting of refringent granules, and a central one of an alveolar nature—the two zones being separated by a layer of large chromatin granules. The majority of the amœbæ have a single nucleus, but there occur forms which are multinucleate.

Multiplication takes place by binary fission (Fig. 110, 2 and 3). In nuclear division the large chromatin granules separating the two nuclear zones are replaced by smaller granules, and these arrange themselves in the form of a band across the nucleus, which becomes elongated. The band then divides into two clusters of chromatin granules, which pass to opposite poles of the nucleus. The latter now becomes hour-glass-shaped, and finally divided into two. The chromatin of each daughter nucleus then arranges itself as granules between the two zones, as occurred in the parent nucleus. Division of the cytoplasm then takes place.

After a number of divisions of this kind, according to Mercier (1910) a sexual phase is initiated. The amœbæ which are to enter on this stage of development are 40 to 50 microns in diameter, and though the cytoplasm is highly vacuolated there are no food inclusions. The nucleus, which at first has the structure described above, changes its character. Some of the chromatin in the intermediate zone is extruded from the nucleus, while the nuclear membrane becomes much thinner. At the centre there appears a large karyosome made up of an achromatic material impregnated with chromatin granules, while a centriole can be detected at the centre. The nucleus elongates, and from the karyosome there is formed an intranuclear spindle with a centriole at each pole. The chromatin granules upon the spindle fibres become separated into two groups, which collect at each pole of the spindle. The nucleus then divides by constriction. By repeated divisions of this kind, eight nuclei are ultimately formed (Fig. 110, 4-7). The cytoplasm now becomes separated into an outer clear alveolar layer and a central granular portion which contains the nuclei, and this is followed by the formation of a cyst wall. The cyst has a diameter of 30 to 50 microns. After encystment, a second period of nuclear multiplication occurs, with the result that as many as sixty nuclei may be formed. The number of nuclei varies considerably (Fig. 110, 7-9). The cysts at this stage escape from the intestine and are taken up by other cockroaches.

In the crop the cyst wall becomes thin, and the centrally arranged nuclei now take up a position at the periphery. The cyst then passes into the mid-gut, where it ruptures and liberates the multinucleate cytoplasmic body (Fig. 110, 10). A process of budding then occurs, by which small uninucleate amœbæ are separated. These buds are supposed to be gametes which unite in pairs, giving rise to zygotes, which gradually increase in size and grow into the free-living adult forms (Fig. 110, 11-23).

The cycle of development is essentially the same as that of *Entamœba coli.* A phase of multiplication by binary fission in the gut is succeeded by encystment. In the case of *E. blattæ*, nuclear multiplication commences before encystment actually takes place, and is continued after the cyst

wall has formed till large numbers of nuclei occur, while in *E. coli* nuclear multiplication begins only after encystment, and there are rarely more than eight nuclei. In the case of *E. coli* the fate of the amœbæ which emerge from the cyst in the new host is not known, and it is possible that

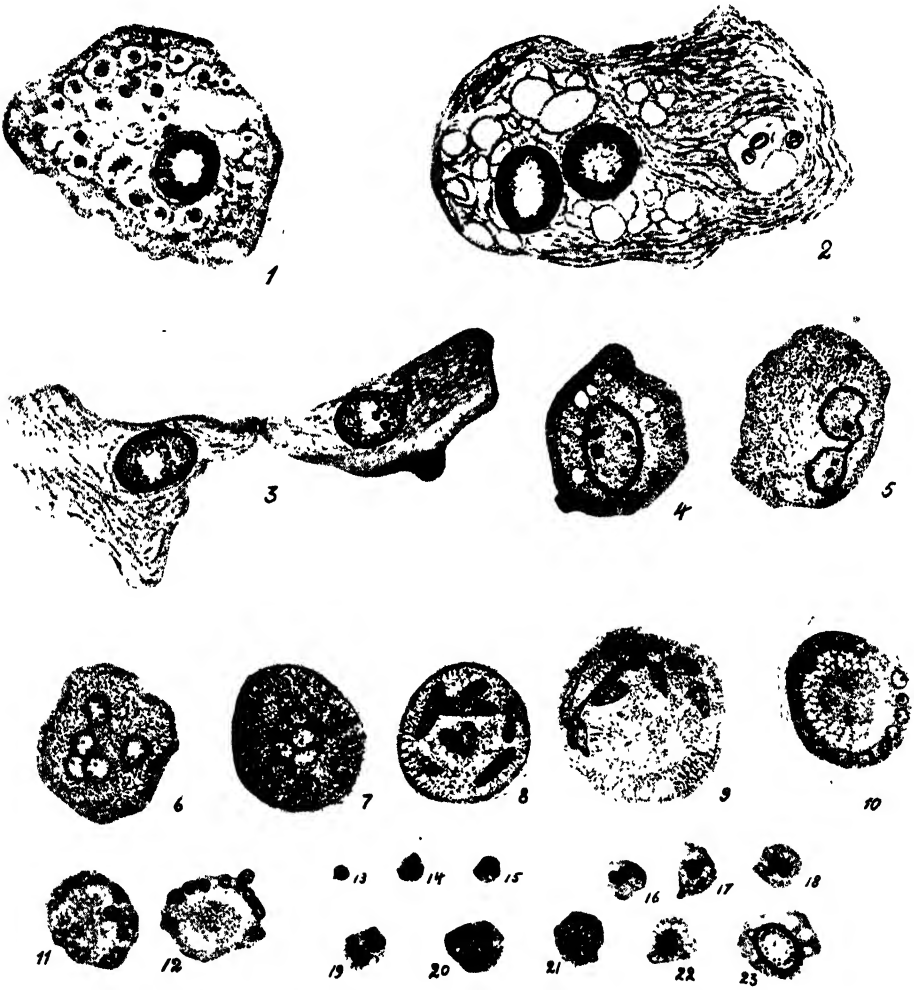


FIG. 110.—*Endamœba blattæ* FROM THE INTESTINE OF THE COCKROACH ($\times$ 600). (AFTER MERCIER, 1910.)

1. Amœba with characteristic nucleus and many vacuolic inclusions.
2. Amœba after division of nucleus. 3. Amœba showing final stage of division.
4-6. Multiplication of nuclei in preparation for encystment.
7-9. Encysted forms: nuclei multiplying.
10. Escape of multinucleated amœba from cyst.
11-12. Production of gametes from multinucleated amœba. 13-15. Free gametes.
16-18. Stages in union of gametes. 19-23. Stages in growth of zygote.

eight small amœbæ are formed, and that these conjugate as in *E. blattæ*, but there is as yet no evidence that this occurs. It is unfortunate that Mercier's observations have not been confirmed, and till this has been done some caution must be shown in accepting his account as absolutely correct.

Genus: **Endolimax** Kuenen and Swellengrebel, 1917.

The genus includes parasitic amœbæ of small size, each of which has a single nucleus with a relatively large karyosome of irregular shape. The cysts are spherical, ovoid, or more irregular in shape, and possess one, two, four, and more rarely eight nuclei. The genus was created by Kuenen and Swellengrebel for a small amœba of the human intestine, which was named *Entamœba nana* by the writer and O'Connor (1917). Boeck and Stiles (1923) believe that the genus is not sufficiently defined, and that it might be better to suppress it as a synonym of *Entamœba*, or to regard it as a sub-genus of *Entamœba*.

ENDOLIMAX OF MAN.

Endolimax nana (Wenyon and O'Connor, 1917).—*Synonymy.*—The writer and O'Connor (1917), who first described this amœba, named it *Entamœba nana*, while later in the year Kuenen and Swellengrebel (1917), who also discovered it, employed the name *Endolimax intestinalis*. Brug (1917) pointed out that it could not be included in the genus *Entamœba*, chiefly because of its distinctive nuclear structure, and placed it in the genus *Vahlkampfia*. Finally Brug (1918) realized that it did not belong to the genus *Vahlkampfia*, and, accepting Kuenen and Swellengrebel's generic title, named it *Endolimax nana*, by which name it is now generally known, though Brumpt (1922) refers to it as *Endolimax phagocytoides*, assuming that an amœba cultivated from human fæces by Gauducheau (1907, 1908), and named *Entamœba phagocytoides*, was actually *E. nana*.

Endolimax nana is one of the commonest protozoa of the human intestine. It was seen by the writer in 1912, and by other observers before and after this, but its true nature was not recognized. The writer and O'Connor (1917) found it to be very common in persons in Egypt.

In the free condition *E. nana* measures from 6 to 12 microns in diameter (Fig. 111). As usually seen, it moves in a sluggish manner, but it may be quite active when observed on the warm stage. When at rest a superficial layer of clear cytoplasm can be distinguished from a vacuolated endoplasm, but when it performs amœboid movements little, clear, blunt pseudopodia are formed. The food vacuoles contain bacteria. The nucleus is detected with difficulty in the living organism, so that if the characteristic cysts cannot be found in any specimen of fæces it is often necessary to prepare stained films in order to distinguish the amœba from the small precystic form of *E. histolytica*. The nucleus is a vesicular structure, and has a diameter of 2 to 3 microns. There is a definite

nuclear membrane which appears to be free from chromatin, all of which seems to be concentrated in the karyosome. The latter varies very much in shape, and may consist of an aggregation of several distinct granules. There may be a single angular or irregular mass more or less central in position: or lying against the nuclear membrane on one side there may be a large mass connected by a fibre with a smaller mass at the opposite side. In other nuclei a single large mass may be connected with two or

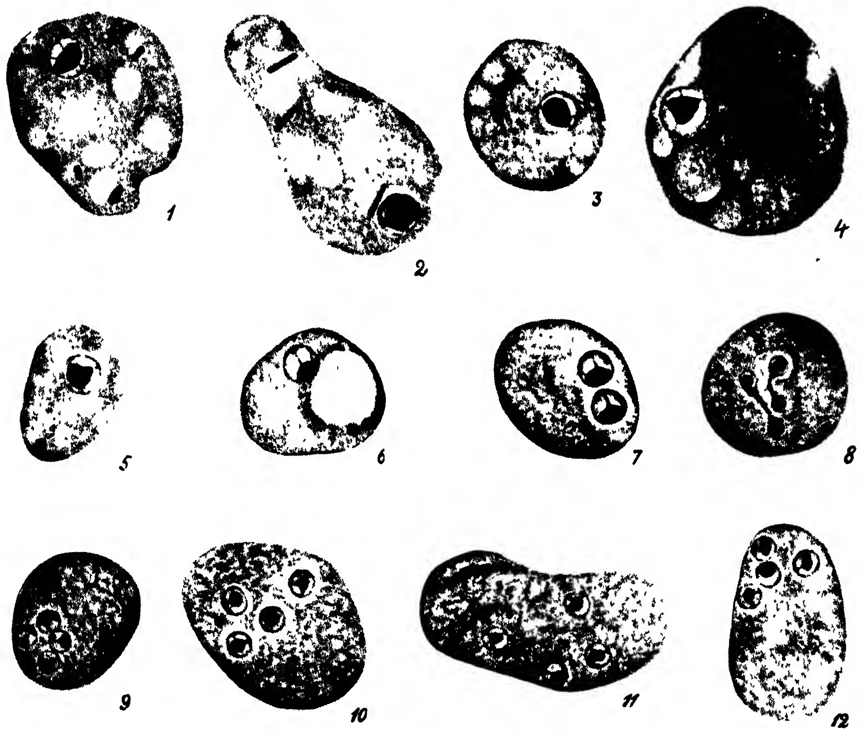


FIG. 111.—*Endolimax nana* (× 3,000). (ORIGINAL.)

1-3. Vegetative forms. 4. Vegetative forms parasitized by *Sphærita*. 5-6. Uninucleated cysts, one with glycogenic vacuole. 7. Cyst with two nuclei. 8. Cyst with two nuclei in division. 9-12. Cysts with four nuclei.

more smaller granules. The karyosome in its typical form is not a spherical body, like the karyosomes of the small amœbæ (*Hartmannella*), which frequently develop in old material from cysts which have passed through the intestine. It is the marked irregularity in the shape and structure of the karyosome which is such a characteristic feature of *E. nana*, and enables it to be distinguished from the small forms of *E. histolytica* (Fig. 95, 10 and 11).

The cyst of *E. nana* was first figured by the writer (1915*e*), who then thought it might be a cyst of *Chilomastix mesnili*. This error was sub-

sequently corrected by the writer and O'Connor (1917). The cyst, which has four nuclei in the mature condition, is typically ovoid in shape, and measures from 8 to 10 microns in length and about half this in breadth (Fig. 111, 5-12). As a rule, one side is less convex than the other, so that the outline is not quite symmetrical. Sometimes the cysts are spherical, and between these and the typically ovoid forms various gradations occur. The nuclei in the cysts are constituted similarly to those of the amœbæ, but in the four-nuclear cysts they are very minute. A characteristic picture is that of a large chromatin body on one side of the nucleus connected to a smaller body at the other side, as sometimes occurs in the free forms.

Attention has been drawn by Dobell (1919) to the occasional presence in the cytoplasm of a glycogen vacuole (Plate II., 22, p. 250). In some batches of these amœbæ a large proportion of the uninucleate cysts possess such a vacuole, while in others it is not so apparent. As noted by Swellengrebel and Winoto (1917), the glycogen gradually disappears in cysts kept for some days outside the body. Dobell has noted the occasional presence of eight instead of the usual four nuclei within the cyst. The writer has also seen these forms. The cysts may contain certain filamentous bodies the nature of which is not clear. Dobell suggests they may be parasitic or symbiotic bacteria, or possibly chromatoid bodies.

In fresh saline preparations the cysts appear as perfectly clear homogeneous structures. The nuclei can rarely be detected, and even in iodine solution they are often difficult to see (Plate II., 17-22, p. 250). In specimens stained with iron hæmatoxylin they are generally quite evident, but it is difficult to gauge the exact degree of differentiation on account of the small size of the cysts. The nuclei may occupy any position in the cyst, but not infrequently they are grouped at one end.

E. nana is an inhabitant of the large intestine, and the writer (1920), in sections of the large intestine, has noted the presence of these amœbæ in the lumen of the glands. There was no evidence that they could invade the tissues. Whether the amœbæ can also live in the small intestine, as Dobell (1919) conjectures, is not known. There is no indication that *E. nana* is in any way pathogenic, and in this respect it resembles the harmless *E. coli*.

Attempts at cultivation of *E. nana* on solid media have not met with success. If fæces containing them are smeared on the surface of suitable agar medium, cultures of small amœbæ of the same size may be obtained, but these are merely developed from cysts of free-living forms.

Several observers have undoubtedly seen *E. nana* in human fæces, and have thought they have obtained cultures of it in agar plates. It is probable that as the *E. nana* perished the cysts of free-living amœbæ gave rise to a culture, and produced an erroneous impression of culture

of *E. nana*. The amœba cultivated by Gauducheau (1907, 1908), and named by him (1907) *E. phagocytoides*, was probably *E. nana* in the fresh stool, but a free-living amœba in the culture. A further paper published by him (1922) tends to confirm this opinion. As his description undoubtedly applied chiefly to the cultivated form, it seems inadmissible to employ his specific name *phagocytoides* for the human parasite, as Brumpt (1922) and others have done. Thomson and Robertson (1925) have maintained a strain of *E. nana* in Boeck and Drbohlav's L.E.A. medium for nineteen days, during which fifteen subcultures were made.

Kessel (1923*a*, 1924*a*) states that he has succeeded in infecting rats and monkeys with *E. nana*. Chiang (1925) was unable to confirm these observations on rats.

ENDOLIMAX OF ANIMALS.

Minchin (1910*a*) described as *Malpighiella refringens* a parasite he had encountered in the Malpighian tubes of rat fleas (*Ceratophyllus fasciatus*). It had an amœboid phase, and also produced cysts which

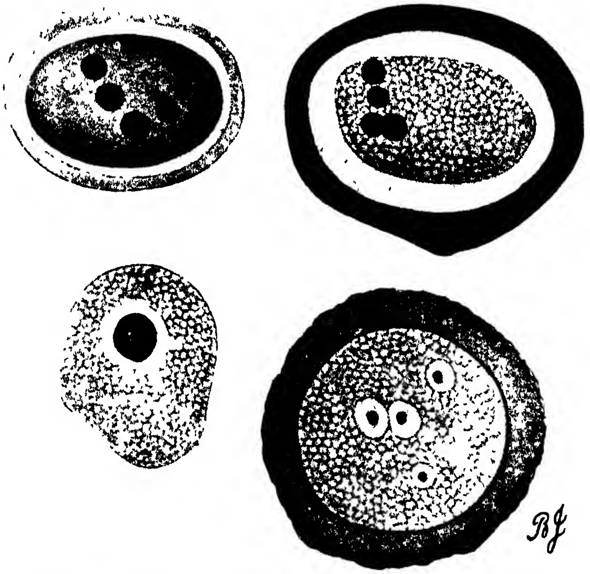


FIG. 112.—*Malpighiella refringens* FROM THE MALPIGHIAN TUBES OF THE RAT FLEA, *Ceratophyllus fasciatus*: AMŒBOID AND THREE ENCYSTED FORMS (× *ca.* 3,000). (AFTER MINCHIN, 1910, FROM DOFLEIN, 1916.)

resemble both in size and appearance those of *E. nana* (Fig. 112). The cyst wall, however, is much thicker than that of *E. nana*. Nöller (1914) observed the organism in the Malpighian tubes of about 90 per cent. of the dog fleas (*Ctenocephalus canis*) in Germany. From the fact that

the unencysted stages do not ingest solid food, he doubts if the parasite is in reality an amœba at all. A very similar parasite was seen by Alexeieff (1913) in the vagina of the leech (*Hirudo medicinalis*). Dobell (1919) thinks that if *Malpighiella refringens* ultimately proves to be an amœba, *E. nana* may have to be placed in the genus *Malpighiella*. An amœba, described by Epstein and Ilovaiski (1914) as *Nægleria ranarum*, from the frog probably belongs to the genus *Endolimax* on account of the structure of the nuclei, and the encysted forms which resemble those of *E. nana*. The free amœbæ reached a diameter of 25 microns (Fig. 113).

Tyzzer (1920) described a small amœba which he found in the intestine of fowls in America. In the free state it resembled *E. nana*, but the central karyosome of the nucleus was more compact. Cysts with a single nucleus

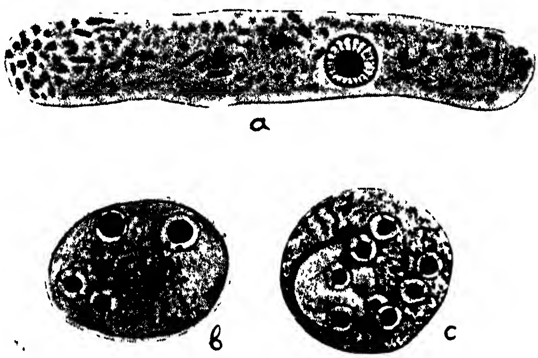


FIG. 113.—*Endolimax ranarum* FROM THE RECTUM OF THE FROG (× *ca.* 1,200). (AFTER EPSTEIN AND ILOVAISKI, 1914.)

a. Free form. *b.* Encysted form with four nuclei. *c.* Encysted form with eight nuclei.

were seen. Though the name *Pygolimax gregariniformis* was given to the amœba on account of its peculiar gregariniform movements, it possibly belongs to the genus *Endolimax*.

Brug (1923), in Sumatra, has seen in the monkey, *Macacus cynomolgus*, an amœba which in its free and encysted stages corresponds with *E. nana*. He names the amœba *Endolimax cynomolgi*. Chiang (1925) has given the name *Endolimax ratti* to an amœba of the white rat. It is morphologically identical with *E. nana*, with which he was unable to infect rats.

Genus: **Iodamœba** Dobell, 1919.

This genus was founded by Dobell for an amœba of the human intestine which produces a uninucleated cyst. The latter contains a very distinct, sharply-defined iodophilic body of glycogen nature which stains a dark reddish-brown in iodine solution. Because of the presence of this idio-

philic body the writer (1915*e*, 1916), who first described it, called it an "iodine cyst," as its exact nature was not clear. Both the cysts and amœbæ were found in a case by Kuenen and Swellengrebel (1917), and Brug (1919) came to the conclusion that the amœbæ seen by Kuenen and Swellengrebel, and which they had called "Pseudolimax," were in reality amœbæ of which the "iodine cyst" represented the encysted stage. Kofoid, Kornhauser and Swezy (1919), and Brug (1921), believe that *I. bütschlii* is a large race of *E. nana*, and express the opinion that *Iodamœba* is a synonym of *Endolimax*, and Boeck and Stiles (1923) support them in this conclusion. It seems, however, that the genus *Iodamœba* is much better defined than these observers maintain.

IODAMŒBA OF MAN.

Iodamœba bütschlii (Prowazek, 1912)—*Synonymy*.—There seems to be considerable doubt as to the correct name of this amœba. Prowazek (1912*a*) gave a very brief and incomplete description of an amœba which he saw in a child in the Caroline Islands. He gave it the name *Entamœba bütschlii*. A single cyst is figured, and if it represents one of the "iodine cysts" it is evidently deformed or degenerate. Dobell (1919) comes to the conclusion that Prowazek was actually describing the "iodine cyst" and its amœboid stage, and that the human parasite should therefore be known as *Iodamœba bütschlii*. It is quite evident that the figures given by Prowazek cannot represent either *E. coli* or *E. histolytica*. The size of the amœbæ excludes the possibility of its being *Endolimax nana*, and from what is now known of the intestinal amœbæ of man the only amœba which Prowazek could have observed is the one now under discussion. On the other hand, Brug (1921) believes that another amœba, previously described by Prowazek (1911, 1912) as *Entamœba williamsi*, was a mixture of the "iodine cyst" and *Entamœba coli*. In support of this contention he states that he has examined Prowazek's original preparations, and has seen in them the iodine cysts and the amœba, an observation which has also been made by Nöller (1921). There can be no doubt, however, that Prowazek's description and figures were based chiefly on *Entamœba coli*, and though some of the forms described by him may have been other amœbæ, the name *E. williamsi* must become a synonym of *E. coli*. The fact that Brug and Nöller have found the "iodine cyst" and its amœba in the original preparations does not prove that Prowazek actually described them. Taliaferro and Becker (1922) support Brug and Nöller in their contention that the correct specific name must be *williamsi*. Brug further considers that the amœbæ belong to the same genus as *Endolimax nana*, while Kofoid, Kornhauser, and Swezy (1919) concluded that they are merely large races of *Endolimax nana*. Rodenhuis (1919) also expressed the opinion that the amœba belonged to the genus *Endolimax*, and proposed to name it *Endolimax pileonucleatus*. Cauchemez (1921) has studied this organism, and, in agreement with Brumpt, comes to the conclusion that it cannot be identified with either of Prowazek's amœbæ, *E. williamsi* or *E. bütschlii*, and proposes to name it *Iodamœba wenyoni*, Brumpt, 1921. This is undoubtedly incorrect, for if it is necessary to reject both of Prowazek's names, the correct name will be *Iodamœba pileonucleata*. It seems, therefore, best to consider the organism as identical with Prowazek's *E. bütschlii*, and to name it *Iodamœba bütschlii*, as Dobell (1919) has done. Kuenen and Swellengrebel (1917) used the name "Pseudolimax," but not as a generic title, though Brumpt (1922) has adopted it as the generic name for this amœba, to which he refers as *Pseudolimax wenyoni*.

The free forms of *I. bütschlii* are intermediate in size between those of *Entamœba coli* and *Endolimax nana* (Fig. 114, 1-4). They are 9 to 13 microns in diameter, but larger forms up to 20 microns and smaller ones down to 5 microns in diameter have been seen. Kuenen and Swellengrebel (1917), who first described the amœba, gave 10 to 12 microns as the measurement, while Brug (1921) gives 7 to 20 microns. Taliaferro and Becker (1922) state that the largest form seen by them measured 20 by 15 microns. There is no marked distinction of ectoplasm and endoplasm, and the movements are sluggish, like those of *E. coli*. The endoplasm contains numerous food vacuoles, which include various bacteria. According to Brug, the amœba feeds only on very small particles, and does not ingest large bodies, as *E. coli* often does. In the living amœba the nucleus can hardly be detected, a feature which serves to distinguish it from *E. coli*, the nucleus of which is nearly always distinct. As first pointed out by Dobell (1919), and later by Taliaferro and Becker (1922), it is the structure of the nucleus which is the most characteristic feature of the free forms. As seen in stained specimens, it is a vesicular structure with a diameter of 2 to 3·5 microns. There is a large karyosome, which has a diameter of about a third to a half of that of the nucleus itself. The membrane of the nucleus is well developed, while the karyosome is surrounded by a layer of globules composed of a substance which does not retain the stain as long as the karyosome, and is thus probably not of chromatin nature. These globules sometimes indent the karyosome, and give it a stellate appearance, while the septa between the globules may produce the impression of a series of radiating fibres connecting the karyosome to the nuclear membrane. Multiplication by binary fission has been noted by Rodenhuis (1919), but the details of the process have not been described. Amœbæ with cytoplasm devoid of good vacuoles, and with or without a glycogenic body, are probably precystic forms (Fig. 114, 5).

The cysts of *I. bütschlii* were first seen by the writer in 1906 in the Sudan, and were not seen again till 1915, when a description was given. They appear to be much more frequently encountered in stools than the free amœbæ, and as very heavy infections sometimes occur without it being possible to discover any amœbæ, the writer considered that the cysts might be vegetable organisms. This view seemed to receive support from the fact that filaments grew out from certain cysts when kept under observation in saline solution. It seems clear from what is known now that those cysts which produced filaments were really of another nature, and not the cysts of the amœbæ. The writer has on several occasions kept cysts which were not identifiable, and has seen them produce long branching filaments across the preparations, a clear indication that they were spores of fungi.

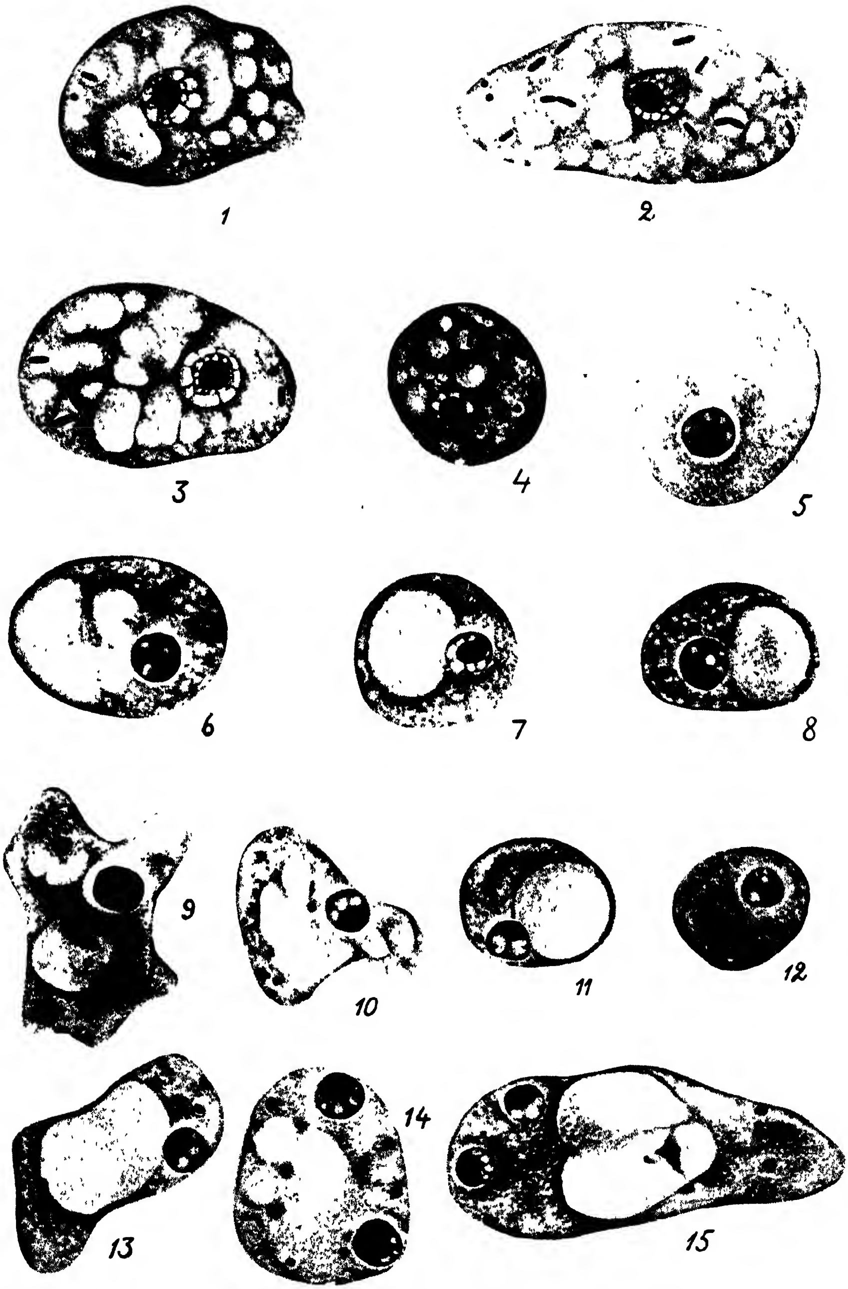


FIG. 114.—*Iodamœba bütschlii* FROM THE HUMAN INTESTINE (× 3,000). (ORIGINAL.)

1-4. Ordinary type of free form.
5. Precystic form.
6-13. Ordinary type of encysted form.
14-15. Encysted forms with two nuclei.

The cysts of *I. bütschlii*, when spherical, vary in diameter from 7 to 15 microns, but very marked irregularities in shape occur (Fig. 114, 6-15). There is a definite cyst wall, and in the cytoplasm within the cyst is found a more or less rounded refractile body and a number of small refractile granules which are possibly composed of volutin. The single nucleus can usually be detected in the thickest portion of the cytoplasm, between the refractile body and the cyst wall. In iodine solution the refractile body assumes a dark brown colour, and is seen to have a sharply-defined margin, thus contrasting with the ill-defined limits of the glycogenic vacuoles in cysts of *Entamœba coli* and *E. histolytica* (Plate II., 11-16, p. 250). The "iodophilic body" is rarely absent from the cysts. It may be quite small, but usually has a diameter of a quarter to a third of that of the cyst. Occasionally two or three separate iodophilic bodies are present. In the process of staining with iron hæmatoxylin and mounting in balsam in the ordinary manner they are dissolved, the vacuoles alone remaining. As pointed out by Dobell, the iodophilic body is gradually absorbed in living cysts kept outside the host. Usually there is a single nucleus in the cysts, though cysts with two nuclei are not uncommon in some infections (Fig. 114, 14 and 15). Taliaferro and Becker (1922) found only four cysts with two nuclei amongst 2,000 consecutive cysts examined. The nucleus of the encysted form differs from that of the free amœbæ in that the karyosome, instead of occupying a central position, comes to lie against the nuclear membrane, while the rest of the space within the membrane is filled with the globules which surrounded the karyosome in the free amœba. These may retain the stain irregularly, and give rise to the appearance of secondary karyosomes in the nucleus. Brug (1919 and 1921) describes the karyosome as being applied to the nuclear membrane, while between it and the opposite side of the nucleus is a body which is semilunar in outline in side view, or watch-glass in shape, with the karyosome at the centre, when viewed in a direction at right angles to this. It is possible that this appearance is a result of shrinkage of the globular material filling the space within the nuclear membrane, so that it forms a more compact body separated from the karyosome and nuclear membrane by a clear space. The karyosome does not always stain uniformly, as often a more deeply staining portion can be distinguished from another staining less intensely.

Dobell (1919) believes that there occur races of *I. bütschlii* which can be distinguished by the average size of the cysts.

I. bütschlii is a fairly common inhabitant of the human intestine. A remarkable feature of the infections is that often enormous numbers of cysts are passed without there being any indication of the free forms. There seems to be no evidence that the amœba has any pathogenic

properties. Kessel (1923*a*, 1924*a*) states that he has infected rats and monkeys with *I. bütschlii.*

On two occasions, by inoculating Boeck and Drbohlov's medium with fæces containing cysts of *I. bütschlii*, Thomson and Robertson (1925) obtained cultures of amœbæ which appeared to belong to this species. One strain was maintained for forty-six days with forty subcultures. No cysts were found in the cultures.

IODAMŒBA OF ANIMALS.

O'Connor (1920) describes an amœba which he found in pigs in the Ellice Islands. Both free and encysted forms occurred, and save for the presence of numerous irregular bodies of chromatoid or volutin nature,

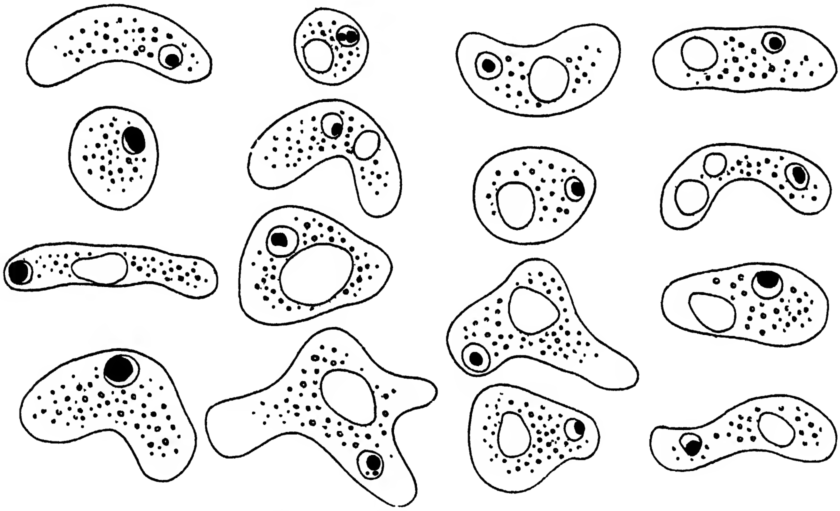


FIG. 115.—CYSTS OF *Iodamœba* OF THE PIG ($\times$ *ca.* 1,400), DRAWN FROM AN IODINE PREPARATION, SHOWING MARKED VARIATION IN SHAPE AND ABSENCE OF IODOPHILIC BODY IN SOME CYSTS. (ORIGINAL.)

they bore a striking resemblance to *I. bütschlii.* The name *I. suis* was suggested, though no data for distinguishing it from the human parasite were given. Nöller (1921), who has studied several cases of infection with this amœba in men in Hamburg, stated that Feibel has noted that at least 20 per cent. of the pigs slaughtered in Hamburg abattoirs had *I. bütschlii* in their intestines. In the same year Cauchemez discovered the organism in pigs in France. He concludes that these animals are probably the reservoirs from which human beings become infected. Feibel (1922) has given an account of the observations referred to by Nöller. The writer has on several occasions seen the cysts of this parasite in fæces of pigs in England (Fig. 115).

Brug (1920*a*) described as *Endolimax kueneni* an amœba he met with in the monkey, *Macacus cynomolgus*. The amœbæ were 7 to 12 microns and cysts 7 to 10 microns in diameter. The latter closely resembled the cysts of *I. bütschlii*, and it is evident this monkey amœba belongs to the same genus, its name becoming *I. keuneni* (Brug, 1920). Hegner and Taliaferro (1924) state that they have seen what appears to be the same parasite in the Brazilian monkey, *Cebus variegatus*, while the writer has seen the cysts of a similar form in the fæces of a gorilla.

Genus: **Dientamœba** Jepps and Dobell, 1918.

The genus includes small, delicate, actively motile, parasitic amœbæ, which show a tendency to remain in a binucleate condition. The nucleus

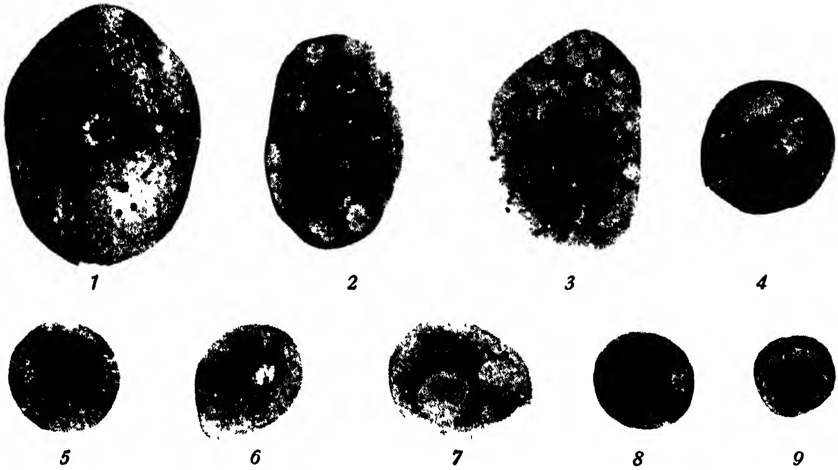


FIG. 116.—*Dientamœba fragilis* (× 3,000). (ORIGINAL.)

1. Vegetative form with two nuclei.
2. Vegetative form with two nuclei, one of which has the chromatin on the nuclear membrane.
3-5. Forms with two nuclei. 6-9. Forms with one nucleus.

has a characteristic structure, and encysted forms have been once recorded. The single known species of this genus was described by Jepps and Dobell (1918) as a parasite of man. They noted eight cases, while Jepps (1921) mentioned ten others in England. The writer saw this form in 1909, but at that time formed no opinion as to its nature. It has been recorded in America by Kofoid, Kornhauser, and Plate (1919), and by Taliaferro and Becker (1922*a*, 1924); in Manila by Haughwout and Horrilleno (1920), both in children and adults; by Bijlsma (1919) in Holland; by Nöller (1921) in Hamburg; and by Thomson, J. G. and Robertson (1923), and Robertson (1923) in England. Reichenow (1923) examined the stools of 100 patients in Germany, and found the amœba in five of these,

Dientamœba fragilis, Jepps and Dobell, 1918.—This is a small amœba which has been seen chiefly in the unencysted stage (Fig. 116, 1-9). It measures 3·5 to 12 microns in diameter. The amœbæ are actively motile, and have a well-marked ectoplasm and endoplasm. The pseudopodia are composed almost entirely of ectoplasm, and these are often flattened or lobed. The endoplasm contains numerous food vacuoles in which bacteria occur. The most characteristic feature of this amœba is its binucleate condition. The nuclei vary in size from 0·8 to 2·3 microns, and are exceedingly difficult to detect in the living organism. In stained specimens the nuclei are seen to be spherical, while there is a central karyosome consisting of a group of granules embedded in a plastin matrix. One granule is generally larger than the others. Surrounding this karyosome is a clear area limited by a fine nuclear membrane, which is connected with the karyosome by exceedingly delicate threads. At the point of union of the latter with the nuclear membrane there are certain granules, which may or may not be chromatin. Apart from these, all the chromatin of the nucleus is aggregated in the karyosome. Though the nuclear structure described above is characteristic of the majority of amœbæ in any one case, the writer has noted that very frequently there is a different arrangement of the chromatin. In some forms it appears to be distributed on the inner surface of the nuclear membrane, while a minute central karyosome can be detected. In other cases, chromatin granules are separated from the membrane, but lie at some distance from the karyosome; while in others they are concentrated at the centre of the nucleus, as Jepps and Dobell state, so that the karyosome may be obscured. Thomson and Robertson (1923) have called attention to the presence of the central karyosome round which the chromatin granules are arranged. It seems doubtful, therefore, if the aggregation of granules at the centre of the nucleus should be regarded as the karyosome, which appears to be represented by a minute granule, as in members of the genus *Entamœba.*

Most individuals are binucleate, but a certain number of uninucleate forms can generally be found. Jepps and Dobell believe that the adult binucleate amœba divides to give rise to uninucleate forms, and that as these grow the nucleus divides in preparation for the division of the cytopolasm, which does not take place till much later. *D. fragilis* is a very delicate organism, and quickly degenerates outside the body. In so doing, a large central vacuole often appears, reducing the amœba to a ring of cytoplasm in which the two nuclei remain. A striking resemblance to *Blastocystis* is thus produced (Fig. 118).

No encysted forms were discovered by Jepps and Dobell, and the writer and others have similarly failed to find any indication of encystment

in cases studied by them. Kofoid (1923), however, describes spherical cysts of this amœba containing one or two nuclei and one or more vacuoles.

In a case seen by the writer, in which *E. histolytica* as well as *D. fragilis* occurred, both infections disappeared after a course of emetine.

Thomson and Robertson (1925) report the successful culture of *D. fragilis* in the medium used by Boeck and Drbohlav for the culture of *E. histolytica*.

DIAGNOSIS OF THE INTESTINAL AMŒBÆ OF MAN.

In order to arrive at a conclusion regarding the nature of the amœbæ or their cysts which are found in human fæces, very careful, and sometimes prolonged, examinations are necessary. It must always be remembered that mixed infections are common, so that it is never possible to be absolutely certain that all the amœbæ present have been diagnosed. In the case of malaria there may be found a large infection of ring forms about the nature of which there may be considerable doubt. Search may reveal a few crescents, and though it will then be known that *Plasmodium falciparum* is present, it will still be impossible to assert that all the rings belong to this species. Similarly, with the amœbæ there may be a mixture of free forms of *E. coli* and *E. histolytica*, and the discovery of the characteristic cyst of one species does not exclude the possibility of some of the free forms belonging to the other (Fig. 117).

Examinations repeated on several different occasions reduce this error to a minimum, but cannot entirely eliminate it. It was demonstrated by the writer and O'Connor (1917) in a series of cases that the positive findings which result from the examination of the first specimen yield only one-third of the positive results obtained by examinations repeated on a number of successive days. Very often it may be impossible to determine the nature of the free amœbæ in any specimen. The large forms may be *E. coli* or *E. histolytica*. The intermediate forms may be either of these or *I. bütschlii*, while the small forms may be any of these or *E. nana* or *D. fragilis*. The precystic forms of *E. coli* and *E. histolytica* may be difficult to distinguish from one another. Diagnosis is most easily made by finding the cysts in saline or iodine preparations, if not on one day, then on another. If cases are examined repeatedly, encysted forms will generally be found (Plate II., p. 250).

As regards the large amœbæ from 15 to 20 microns or more, if they occur in dysenteric stools and are very active, they are probably *E. histolytica*. If so, the nucleus should be difficult to see, and search may reveal forms including red blood-corpuscles. In the latter case the amœbæ are certainly *E. histolytica*. *E. coli* may, however, be present in dysenteric

PLATE II.

Cysts of various human intestinal Protozoa, as seen in iodine solution (x 2000).

1-4. *Entamœba coli.*
5-10. *Entamœba histolytica.*
11-16. *Iodamœba bütschlii.*
17-22. *Endolimax nana.*
23. *Giardia intestinalis.*
24. *Chilomastix mesnili.*

(*Original.*)

[*To face p.* 250.

conditions of a bacillary nature. The amœbæ are less active, the nuclei are clearly visible, while food vacuoles containing bacteria and other objects are present. In cases of doubt it is necessary to wait till formed

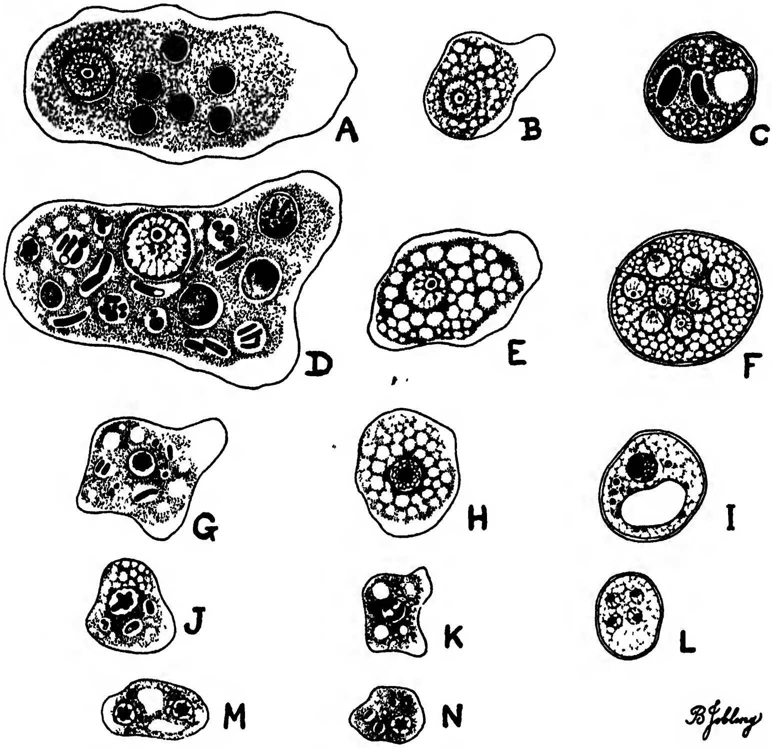


FIG. 117.—THE INTESTINAL AMŒBÆ OF MAN (× 1,250). (AFTER WENYON, 1922.)

A-C. *Entamœba histolytica.*

A. Tissue-invading form with one nucleus and six ingested red blood-corpuscles.
B. Precystic amœba. C. Cyst with four nuclei and chromatoid bodies.

D-F. *Entamœba coli.*

D. Large amœba with one nucleus and various ingested food bodies.
E. Precystic amœba. F. Cyst with eight nuclei.

G-I. *Iodamœba butschlii.*

G. Free amœba. H. Precystic amœba.
I. Cyst with a single nucleus and glycogenic vacuole.

J-L. *Endolimax nana.*

J. Free amœba. K. Precystic amœba. L. Cyst with four nuclei.

M-N. *Dientamœba fragilis.*

M-N. Forms with one and two nuclei.

stools are being passed, when the characteristic cysts of *E. coli* or *E. histolytica* will probably appear. The former are seen either with eight nuclei or with two nuclei and large central vacuole; while the latter have one, two, or four nuclei, and not infrequently chromatoid bodies and

vacuoles. Examined in iodine solution, the details are more readily seen, for the nuclei of the cysts of *E. histolytica* are not easily distinguished in saline solution. Very active amœbæ with very marked ectoplasm and pseudopodia being formed entirely of ectoplasm, are most probably *E. histolytica.* If the amœbæ are about 10 to 15 microns in diameter, then diagnosis is difficult, and a careful search in saline and iodine solution must be made for cysts of *E. coli*, *E. histolytica*, or *I. bütschlii.* The last is distinguished by its deeply staining iodophilic body and single nucleus; the others by the number of nuclei and presence or absence of chromatoid bodies. If no cysts can be found, it will be necessary to make stained films, when *I. bütschlii* can be recognized by its large central karyosome. If its nucleus has a small karyosome and chromatin granules on the membrane, an amœba may be *E. histolytica* or *E. coli.* Attention to details of the nuclear structure, as described above, may assist in diagnosis, but it must be admitted that there is difficulty in distinguishing the precystic forms of these amœbæ.

If precystic amœbæ occur in any specimen, then it is very unusual for cysts not to be present also, and if they are not found at the first examination, later examinations will almost certainly reveal them.

If the amœbæ are quite small and vary in size from 5 to 10 microns, or a little over this, they may be free forms of *E. nana*, precystic forms of small races of *E. histolytica*, *I. bütschlii*, or *D. fragilis.* Here, again, the discovery of cysts will enable a diagnosis of the first three to be readily made. If cysts cannot be found, then films must be stained. The small forms of *E. histolytica* will show their characteristic nuclei, and *D. fragilis* the two nuclei characteristic of this amœba. Amœbæ with a single nucleus, showing a large, irregularly-shaped karyosome, are almost certainly *E. nana*, though it is just possible they may be difficult to distinguish from *I. bütschlii*, which, however, is rarely seen in the unencysted condition. If *I. bütschlii* is present, its cysts will almost certainly be found and recognized in iodine solution. The cysts of *E. nana* are typically of an ovoid shape, while the small cysts of *E. histolytica* are usually spherical or nearly so. The small cysts of *E. histolytica* often show chromatoid bodies, and the details of the cysts and those of *E. nana* should be quite clear in properly stained films.

It must be remembered that in the great majority of cases a diagnosis can be arrived at by the careful examination of thin saline and iodine preparations, and that stained films are only necessary in exceptional cases or for confirmatory purposes.

The presence in large amœbæ of food vacuoles containing bacteria, yeasts, or other objects, amongst which may be cysts of the intestinal Protozoa, such as those of *E. histolytica*, *Giardia intestinalis*, *Isospora*

belli, is almost conclusive evidence of the amœbæ being *E. coli*. It should be remembered, however, that dying or dead *E. histolytica* may be invaded by bacteria of all kinds, while occasionally a particular type of body, such as the spores of a bacillus, may be taken up by apparently healthy *E. histolytica*. All the intestinal amœbæ of man, as well as the free-living amœbæ, are liable to invasion by the vegetable organism *Sphærita*, a name given to it by Dangeard (1886), who saw it in a Heliozoan. It was seen by the writer (1907) in *E. muris* of mice. It has the appearance of spherical masses of coccus-like bodies which are highly refringent in the living condition. In films stained by iron hæmatoxylin they are black. They occur in vacuoles in the cytoplasm (Fig. 111, 4). A less common parasite of similar appearance is one which occurs within the nuclear membrane. It was named *Nucleophaga* by Dangeard (1896), who saw it in the nuclei of free-living amœbæ. Nöller (1921) has described its occurrence in the nuclei of *E. nana* and *I. bütschlii*.

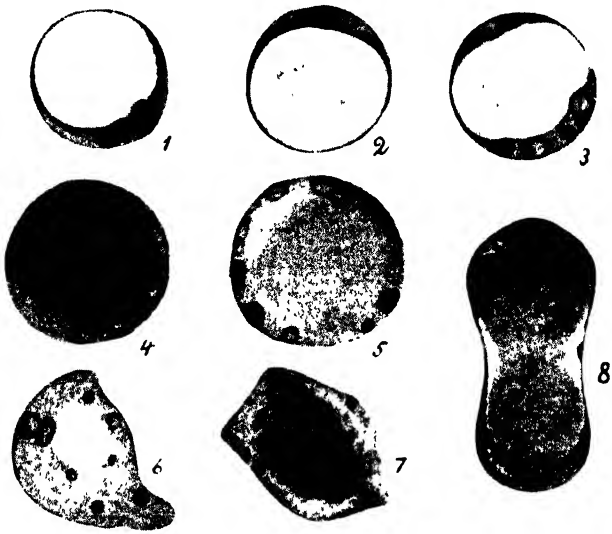


FIG. 118.—*Blastocystis hominis* FROM HUMAN FÆCES FIXED IN SCHAUDINN'S FLUID AND STAINED WITH IRON HÆMATOXYLIN (× 2,000). (ORIGINAL.)

In addition to the nuclei the thin layer of cytoplasm surrounding the large central vacuole contains dark staining granules of volutin. 1-7, Ordinary forms; 8, dividing form.

It is difficult to give any rules for the separation of amœbæ or their cysts from other structures in fæces. *Blastocystis hominis* Brumpt, 1912, is very commonly present, and varies considerably in size (Fig. 118). It has a large central vacuole, while the cytoplasm is reduced to a thin layer in which one or two small nuclei lie at each pole of the cyst. Refractile globules of volutin which may be present in the cytoplasm must not be mistaken for the nuclei, which are much less distinct. The organism has a much more delicate appearance, and is generally less refractile than the amœbic cysts. Usually the central vacuole contains non-refractile material; at other times it contains a highly refractile body which may have a yellowish or brownish tint. *Blastocystis* may

be no more than 5 microns in diameter, or 20 microns or more. In varying number it can be found in practically every stool examined. It reproduces as a rule by binary fission, and multiplies rapidly in certain media, such as that used for the cultivation of *E. histolytica.* Occasionally, as pointed out by Alexeieff (1911*d*), forms with numerous nuclei are seen, and it appears that the cytoplasm concentrates round these nuclei, producing eventually a number of daughter forms within the original cyst membrane. Such a form was seen in human fæces by the writer and O'Connor (1917) in Egypt. *Blastocystis* is a vegetable organism, but not infrequently it may be simulated by cells, flagellates, or amœbæ, which in a degenerating condition develop a large central vacuole. Cysts of amœbæ may be confused with fat globules (castor oil), or globules of semi-digested muscle fibres. The latter may be perfectly spherical and homogeneous. They are usually of a yellow-brown tint. In iodine solution they stain a pale brown colour, or, as sometimes happens, they become definitely pink. They are highly refractile, and show no internal structure either in saline or iodine solutions. When once an observer has become familiar with the actual appearance of the cysts of the intestinal Protozoa, it is hardly possible to confuse them with other objects, and this familiarity can only be obtained by practical experience with the microscope. Intestinal epithelial cells swollen as a result of degeneration, and the large macrophages which are sometimes seen with included red blood-corpuscles, have been frequently mistaken for amœbæ. These cells, however, never exhibit active movements, while their nuclei have an appearance which is quite different from that of the nuclei of amœbæ. As dead and immobile amœbæ may easily be confused with large cells, and *vice versa*, it is safest to regard no cell as an amœba unless definite amœboid movements are seen. Polynuclear leucocytes, in which the nucleus has separated into four parts, may be mistaken for four-nucleated cysts. Occasionally, cysts of free-living Protozoa which have been swallowed in food or water may be met with in perfectly fresh stools. It cannot be too strongly emphasized that specimens examined should be as fresh as possible.

ACTION OF DRUGS ON INTESTINAL AMŒBÆ.

There is only one drug which can claim to have any marked specific action on the intestinal amœbæ of man, and this is *emetine.* Curiously enough, it affects only two of these—namely, *E. histolytica* and *I. bütschlii.* The former is known to be a tissue parasite, while there is no evidence that *I. bütschlii* is anything more than a harmless commensal which lives in the intestinal contents. On *E. coli* and the other forms there is no evidence that emetine has any action whatever.

It has long been known that ipecacuanha is a specific for amœbic dysentery, but Vedder (1912) was the first to show that this action of ipecacuanha depended upon the alkaloid emetine. Rogers (1913), in India, was the first observer to introduce this alkaloid in the routine treatment of amœbic dysentery, a course which had been previously recommended by Vedder (1912). The latter observer believed that it acted directly on the amœbæ and poisoned them, and Rogers made similar claims. It appears, however, as has been demonstrated by the writer and others, that active *E. histolytica*, either in fæces or liver-abscess pus, can be mixed with relatively strong solutions of emetine, and that the amœbæ will remain as perfectly active as those in control preparations. Unless it is assumed that the medium in which the amœbæ happen to be—namely, the fæcal matter or the pus—absorbs or fixes the emetine, so that it never actually comes in contact with the amœbæ, it must be concluded that the alkaloid has no immediate toxic action on the amœbæ. That such an explanation of the failure of emetine to kill amœbæ in these experiments may have something in its favour is borne out by certain tests made by Pyman and the writer (1917) on cultures of free-living amœbæ on agar plates. The agar was made up with varying strengths of different salts of emetin, and it was found that the amœbæ did not grow on the medium which contained the salts, which are known to be specifics for amœbic dysentery, though the bacterial growth upon which the amœbæ feed was little altered in character. Furthermore, it has been shown by Brown (1922) that if the emetine solution which is to be introduced into the agar is first mixed with pus for a few minutes, the liquid portion separated by centrifugation has lost its power of arresting growth of amœbæ on the plate. It would seem that in this experiment the dead cells and débris in the pus had absorbed the emetine from the solution, so that there may be some reason for suspecting that when material such as fæces or pus containing *E. histolytica* is mixed with solutions of emetine, the failure of the drug to kill the amœbæ may be due, in part at least, to its absorption by the dead material. .It has also to be remembered that even if emetine has no direct action on *E. histolytica* exposed to it for a comparatively short time, it may still have such an action over a longer period in preventing growth and multiplication. It should be possible to test this point on cultures of *E. histolytica*.

Dale and Dobell (1917) investigated the action of emetine on experimentally infected cats, and came to the conclusion that the drug only indirectly kills *E. histolytica* by acting primarily on the host. In the case of cats they stated that it neither acts as a prophylactic when given before infection is attempted, nor as a curative agent after *E. histolytica* has established itself in the large intestine. Mayer (1919) had similar experiences,

but Sellards and Leiva (1923) have shown that, as a rule, amœbic dysentery in kittens is so much more acute than it is in human beings that the action of emetine in the two hosts is hardly comparable. By employing large animals, in which the dysentery arising from injection of *E. histolytica* is less acute than it is in kittens, and by treating the animals with emetine solutions *per rectum* in a dose of 10 milligrams per kilogram of body-weight, they have demonstrated a definite therapeutic action of the drug. Furthermore, by employing the same method of treatment in kittens immediately infection has taken place, similar results were sometimes obtained.

Ware (1916) reported an outbreak of what appeared to be amœbic dysentery in a pack of fox-hounds in India. Seven of the animals, some of which had been obstinately ill for several months, were given injections of from ½ to 1 grain of emetine. There was an immediate response with cessation of symptoms. All the animals recovered completely except one, which relapsed. Whatever may be the mechanism of its action, it is certain that in man emetine has a remarkable effect. Attacks of amœbic dysentery are in most cases cut short by the hypodermic injection of 1 grain of the drug on a few successive days, while the introduction of the drug has diminished the number of secondary complications, such as liver abscess, which formerly were of common occurrence.

As in the treatment of so many protozoal diseases (malaria, trypanosomiasis), though it is comparatively easy to suppress the parasites to the extent that acute symptoms disappear, it is extremely difficult to rid the host of *E. histolytica* entirely, and relapses are therefore prone to occur. Very frequently, after the treatment of acute amœbic dysentery, with the disappearance of symptoms the patient passes into the carrier condition. In a certain number of cases it is possible by intensive treatment to rid a patient entirely of an *E. histolytica* infection, but to obtain evidence that this has happened it is necessary to continue the examinations of the stools over a period of many months.

It was shown by the writer and O'Connor (1917) that the administration of 1·5 grains of emetine hydrochloride daily (1 grain subcutaneously each morning and ½ grain by the mouth each night) for a period of twelve days would in a certain number of cases eradicate *E. histolytica* infections. Another method of giving emetine is in the form of the powder of bismuth emetine iodide in cachets. This drug was introduced during the war by Low and Dobell (1916), and has been extensively used. A cachet containing 3 grains of the drug, corresponding to 1 grain of emetine hydrochloride, is given each day for twelve days by the mouth.

Emetine has a remarkable action in cases of threatened amœbic abscess (hepatitis), though it is not quite clear if the drug alone, without operative treatment, will cause an amœbic abscess which has already formed to

disappear, though some observers, such as Rogers, claim that it will. The writer with O'Connor observed a case in Egypt of a liver abscess which was draining after operation. Active amœbæ were constantly present in the pus in spite of the administration of large doses of emetine both subcutaneously, by the mouth, and by injection into the abscess cavity. Here it would seem that failure of the emetine to reach the tissues in which the amœbæ were actually living, possibly as a result of defective circulation, would account for the result.

The action of emetine in getting rid of infection of *I. bütschlii* was first noted by the writer and O'Connor (1917) in Egypt. The same result was obtained by others, as recorded by Dobell, Gettings, Jepps, and Stephens (1918). If it is correct that emetine only acts on *E. histolytica* indirectly by its influence on the tissues of the host, and has no action on *E. coli* and other intestinal amœbæ, it is difficult to understand how it affects *I. bütschlii*, which, as far as we know, is similar in habits to *E. coli*.

In some cases of *E. histolytica* infections emetine fails to act, and in these it can only be supposed that the intestine is in such a condition that the tissues in which the amœbæ are living are not reached by the emetine, possibly as the result of defective circulation in certain portions of semi-necrotic mucosa. The view that emetine resistant strains of *E. histolytica* exist requires definite proof, of which at present there is none, before it can be accepted. There also does not seem to be any evidence that the administration of emetine will cause a sudden encystment of amœbæ in the gut, as has been claimed. When it is understood that encystment is not a simple process, but depends first of all on the production of precystic amœbæ, it is difficult to see how this can be brought about in a short time. It is possible that, if precystic amœbæ are present at the time of administration of emetine, this might accelerate their encystment.

There is no evidence that the cases which resist emetine do so on account of the presence of cysts. Certain resistant cases, as noted by the writer and O'Connor (1917), appear never to pass cysts, free active amœbæ alone being found in the stools whenever these are examined.

AMŒBÆ CULTIVATED FROM FÆCES—COPROZOIC AMŒBÆ.

The fact that amœbæ develop in fæces after they have been passed, and on the surface of agar plates inoculated with fæces, has misled observers into believing that they had been able to cultivate the intestinal amœbæ. Kartulis (1891) claimed to have cultivated amœbæ of the human intestine, but it was pointed out by Celli and Fiocca (1894, 1895), and Casagrandi and Barbagallo (1895, 1897), that the cultures contained

only free-living non-parasitic forms. Musgrave and Clegg (1904, 1906) made extensive observations in Manila. They thought they had cultivated the amœbæ of the human intestine and isolated them from the water supply. They also stated that it was possible to produce dysentery in monkeys by injecting cultures of these amœbæ. The writer (1907), using the same medium, attempted to obtain cultures of *E. muris* of mice and *E. coli* of man, but succeeded in growing only free-living amœbæ. He pointed out that the amœbæ obtained in culture by Musgrave and Clegg in no way resembled *E. coli*, which they claimed to have cultivated. It appeared that what actually happened was that cysts of free-living amœbæ were constantly passing through the intestine of man and animals, and that it was these which were responsible for the cultures obtained. Walker and Sellards (1913) again investigated the claims made by Musgrave and Clegg, and showed that the writer's explanation was undoubtedly correct. By causing individuals to ingest the cysts of the amœbæ which appeared on agar plates, they were able to isolate the same amœbæ a few days later by smearing agar plates with the fæces. Fantham (1911*a*) gave a description of *E. coli* based entirely on agar cultures of free-living amœbæ.

The amœbæ which appear on agar plates after smearing them with the fæces of man or animals are usually small forms which are rarely more than 10 to 20 microns in diameter. They are actively amœboid, and live by ingestion of bacteria which grow at the same time. It is necessary, if good cultures are to be obtained, to have a medium which is not too rich in nutrient material, so that the bacteria do not overgrow the amœbæ. The medium used by Musgrave and Clegg is very suitable, and consists of agar 20 grams, sodium chloride 0·5 gram, extract of beef (Liebig) 0·5 gram, water 1 litre. The solution is then made 1·5 per cent. alkaline to phenolphthalein. About 10 c.c. of the medium is warmed till liquid, and poured into a Petri dish, where it is allowed to set. On this medium with a low power of the microscope the amœbæ may be seen spreading across the surface beyond the edge of the bacterial growth. Multiplication is rapid at laboratory temperature, and in a few days a plate will contain thousands of amœbæ. In the central and older parts of the culture the amœbæ encyst in spherical cysts. Subculture is readily effected by transferring small portions to fresh plates. It is possible, by using a finely-drawn-out glass filament with a rounded bead at the end, under a low power of the microscope, to transfer a single isolated amœba to a new plate, and thus to obtain a perfectly pure culture of a single species.

It has already been mentioned that cultures of amœbæ often appear in stale stools, and care must be taken not to confuse them with *Endolimax*

nana, to which they bear some resemblance. Though these cultural amœbæ resemble one another superficially, they belong to several distinct species. They can be differentiated from one another by a careful study of the nuclear division and the encysted stage. The amœbæ isolated in this way are usually species of *Hartmannella* and *Dimastigamœba*. The shelled form *Chlamydophrys stercorea* and its allies may also occur on agar plate cultures of fæces of animals.

STATISTICS OF INTESTINAL AMŒBÆ OF MAN.

As in the case of most of the parasitic infections of the intestine, the incidence of amœbic infections in any community is directly related to the efficiency or otherwise of the sanitary arrangements. Where there is every possibility of food and water becoming contaminated with fæcal material, either directly, or indirectly by the agency of flies, there the percentage of individuals harbouring intestinal Protozoa will be high. It was shown by the writer and O'Connor (1917) that flies in Egypt are constantly feeding on fæcal material, and that the cysts of intestinal Protozoa, and even the unencysted forms, may quickly pass undamaged through the intestine, and in this way be deposited on food.

It is probably correct to state that all the intestinal amœbæ of man are world-wide in their distribution, the number of individuals actually infected varying with the locality. In tropical countries, where sanitation is generally bad, the incidence is high, while in England it is relatively low, though even here the figures are higher than might be expected. It has already been pointed out that the percentage of infections resulting from a single examination of each case is fallacious, and that repeated examinations usually yield a figure which is at least three times as great as that obtained by a single examination. In Alexandria and London during the war the writer and O'Connor (1917) found the following percentages of infection amongst different groups of men:

	Healthy Troops.	*Convalescents (Alexandria).*	*Convalescents (London).*	*Hospital Cases.*	*Gabarri Prison (British).*	*Hadra Prison (Natives).*	*Native Cooks.*	*British West Indian Cooks.*
Total examined ..	1,979	328	556	961	168	524	87	48
Entamœba histolytica ..	5·3	6·4	10·8	3·2	1·8	13·7	11·5	4·1
Entamœba coli ..	20·0	31·7	39·0	10·4	12·0	48·6	20·7	18·7
***Entamœba* sp. (?)** ..	1·3	1·8	—	2·0	17·2	0·57	1·1	—
Iodamœba bütschlii ..	3·0	2·0	5·2	0·3	—	14·8	7·0	4·1
Endolimax nana ..	0·5	—	1·0	3·0	12·0	—	—	—

The figures given for Hadra Prison show the results obtained by single examinations of natives of the country. In the case of *E. nana*, the low figure is explained by the fact that the examinations were largely made before it was recognized that *E. nana* was a parasitic amœba.

During the war a large number of examinations were made of healthy persons in the British Isles, and though isolated cases of *E. histolytica* infection in individuals who had never left the country had already been recorded by Marshall, D. G. (1912), the writer (1916) and others, it was Yorke and his collaborators (1917) who first showed that amœbic infections were quite common amongst the indigenous population. Dobell (1921) has examined the records of several observers, and after allowing for the errors of the single examination concludes that the percentages of infections to be found amongst the artisan population are as follows: *E. histolytica*, 7 to 10; *E. coli*, 36 to 54; *E. nana*, 9 to 13; *I. bütschlii*, 0·5 to 0·75.

Boeck (1921) has published the results of examination of eighty-three industrial school children in America. Each case was examined, on an average, 5·3 times. He gives the following figures of percentages: *E. histolytica*, 10·8; *E. coli*, 49·3; *E. nana*, 6·0; *I. bütschlii*, 1·2. Similar records have been published from other parts of the world.

2. *Family:* PARAMŒBIDÆ Poche, 1913.

This family includes the single genus *Paramœba*, which was created by Schaudinn (1896) for a marine amœba, *Paramœba eilhardi*, which possessed, in addition to its nucleus, an accessory body (Nebenkörper). Both the nucleus and the "Nebenkörper" divided during division of the amœba. Janicki (1912) pointed out that two amœbæ (*A. pigmentifera* and *A. chætognathi*) which Grassi (1882) had discovered in the body cavity of the small marine worms of the genera *Spadilla* and *Sagitta* belonged to this genus (Fig. 119). During division of the amœba the nucleus divides by mitosis, while the "Nebenkörper" divides by simple elongation and constriction. Small elongate flagellates, each with a single flagellum, are produced. These, after multiplying by division, conjugate and give rise to zygotes which become the amœbæ.

3. *Family:* DIMASTIGAMŒBIDÆ.

This family includes amœbæ which are able, under certain conditions, to develop flagella and behave as flagellates. When they occur in fæces or are cultivated on the surface of agar plates they live and reproduce as amœbæ, but if brought into liquid media they quickly grow flagella and swim about for some hours, after which the flagella are lost and the amœboid phase is resumed. One of these amœbæ was isolated

from human fæces by Schardinger (1899), who named it *Amœba gruberi.* Wasielewski and Hirschfeld (1910) showed that an amœba which they called *A. limax* at certain stages developed two flagella. A similar observation was made by Alexeieff (1912*g*) on an amœba referred to as *A. punctata* (Dangeard). Martin and Lewin (1914) showed that a soil amœba, which they called *Vahlkampfia soli*, readily developed two flagella when an agar plate containing the encysted forms was flooded with tap water containing 0·25 per cent. NaCl and 0·05 per cent. $MgSO_4$. Wherry (1913), working with a similar amœba, could produce the trans-

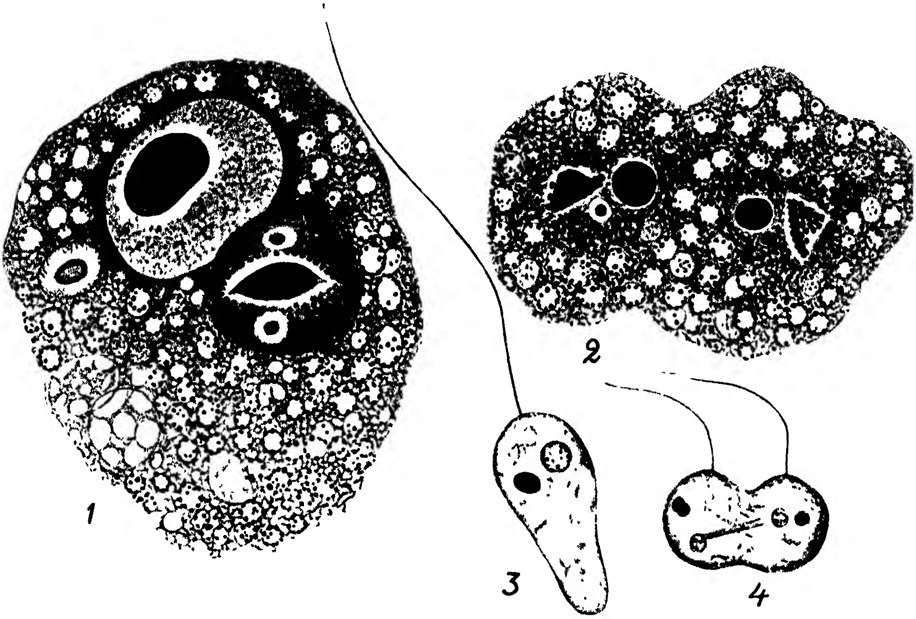


FIG. 119.—PARASITIC AMŒBÆ OF THE GENUS *Paramœba*. (AFTER JANICKI, 1912.)

1. Free form of *P. pigmentifera* with two nuclei (× 1,800).
2. Dividing form of *P. chætognathi* (× 2,700).
3. Flagellate stage of *P. pigmentifera* (× 3,650).
4. Dividing flagellate form of *P. pigmentifera* (× 3,650).

formation by merely diluting a loopful of liquid egg-medium culture of the amœbæ with several loopfuls of distilled water, observations which were confirmed by Wilson (1916). In all these cases the flagellates had two flagella of approximately equal length, and traceable to two blepharoplasts in the cytoplasm or to the nuclear membrane. It is probable that all these amœbæ belong to the species which was first isolated from human fæces by Schardinger (1899), who called it *A. gruberi.* On account of its flagellate stage, it was placed in a new genus, *Nægleria*, by Alexeieff (1912), who later (1912*a*) came to the conclusion that it belonged to the

genus *Dimastigamœba* founded by Blochmann (1894), its correct name being *Dimastigamœba gruberi*. According to Boeck and Stiles (1923), the name *Dimastigamœba* of Blochmann (1894, 1895) refers to another amœba, as also Alexeieff's name *Nægleria*. They adopt the name *Wasielewskia*, proposed by Hartmann and Schüssler (1913), and employed by Zulueta (1917), for the form described here, which they refer to as *Wasielewskia gruberi*. As, however, there is little doubt that the organism described by Whitmore (1911*b*) as *Trimastigamœba philippinensis* is the same amœba, his generic name has priority over all names except *Dimastigamœba*, which nevertheless appears to be the correct name for the genus.

It is evident that *Dimastigamœba gruberi* might be classed with the Mastigophora, instead of with the Rhizopoda. It illustrates very clearly the close relationship of the two groups.

Dimastigamœba gruberi (Schardinger, 1899).—This amœba, which occurs commonly as a coprozoic organism in fæces of human beings and animals, has been studied by the writer in cultures made from dirty water and old fæces. Both on agar plates and in liquid media the organism remains in its amœboid phase, but if sudden changes are made the flagellate phase appears in two to three hours, and lasts up to twenty-four hours or longer than this under exceptional circumstances. Thus, if some of the growth on agar plates is scraped off and mixed with two or three drops of tap water, in two or three hours, according to the temperature, enormous numbers of flagellates are developed. In twenty-four hours they have all reverted to the amœboid form again. A further addition of tap water brings about the reappearance of the flagellate forms. It is quite easy to watch under the microscope the transformation of one of the amœbæ into the flagellate. The amœba becomes rounded, and two flagella commence to grow from the surface of the body. They can be seen to be connected with two small granules, the blepharoplasts, which lie close together on the surface of the body. The nucleus, which is readily seen on account of its large refractile karyosome, may remain near this point, or it may be at some other part of the amœba, or its position may be constantly changing. It has not been possible to observe the origin of the blepharoplasts from the nucleus or its karyosome. They are first detected as such after the flagella have commenced to form. The flagella gradually increase in length, and become more violent in their action. The organism now elongates and becomes pear-shaped, the more pointed end being the flagellar end. The nucleus, if it has not remained near the blepharoplasts during the growth of the flagella, approaches this end of the body. At this stage the typical flagellate is formed. The posterior region of the body is swollen, the anterior being narrow. At one

side of the anterior end can, not infrequently, be made out a slight depression, having the appearance of a small cytostome. The two blepharoplasts, which were first clearly described by Alexeieff (1912*g*), lie one in front of the other on the surface of the cytoplasm within this depression, and the flagella arising from them pass through the opening of the depression. In many individuals a short fibre can be traced from each blepharoplast as far as the nucleus, where it ends in a small thickening or elevation of the nuclear membrane. In some forms the nucleus may be near the centre of the body, or even at the posterior end, and in such cases it may or may not be possible to trace fibres from the blepharoplasts to the nucleus. The nucleus has a large central karyosome, which is connected with the nuclear membrane by radiating filaments. On the inner surface of the membrane are granules of chromatin. The nucleus of the amœboid form is spherical, but in the flagellate phase, in which a connection between the blepharoplasts and nuclear membrane can be made out, the latter structure may be slightly drawn out towards the blepharoplasts. A contractile vacuole is present. In the flagellated forms it is behind the nucleus in the thicker portions of the body. The flagellates, which are typically pear-shaped, vary in length from 10 to 30 microns. The relation of the blepharoplasts to the nucleus are of considerable interest. Alexeieff (1912*g*) stated that when the flagellate phase was to appear, two granules separated from the karyosome and migrated to the surface of the body, retaining in some forms a connection with the karyosome. Wilson (1916) also described the separation from the karyosome of a granule, which migrated into the cytoplasm and became the blepharoplasts. The writer, after examining many thousands of amœbæ at all stages of flagellum formation, has failed entirely to trace the origin of the blepharoplasts from the karyosome. Appearances suggestive of such an origin are occasionally seen, but they are too inconstant to justify the conclusions that the blepharoplasts arise in this manner.

On agar plates there occur amœbæ with one, two, or four nuclei (Fig. 61). Those with one nucleus develop, as a rule, a single pair of flagella (Fig. 120, 1-7); those with two nuclei two pairs (Fig. 120, 13 and 14); and those with four nuclei four pairs (Fig. 120, 15). It is evident, therefore, that each nucleus has associated with it a pair of blepharoplasts. If an amœba has a nucleus in process of division, it will still develop flagella, but in this case two pairs appear, as in the forms with two nuclei (Fig. 120, 11 and 12). It seems evident that with nuclear division the blepharoplasts have divided. In some cases an amœba, with a single nucleus showing no sign of division, will develop two pairs of flagella (Fig. 120, 8-10). It would seem justifiable to conclude that the single pair of blepharoplasts has divided preparatory to nuclear division, which has not as yet visibly

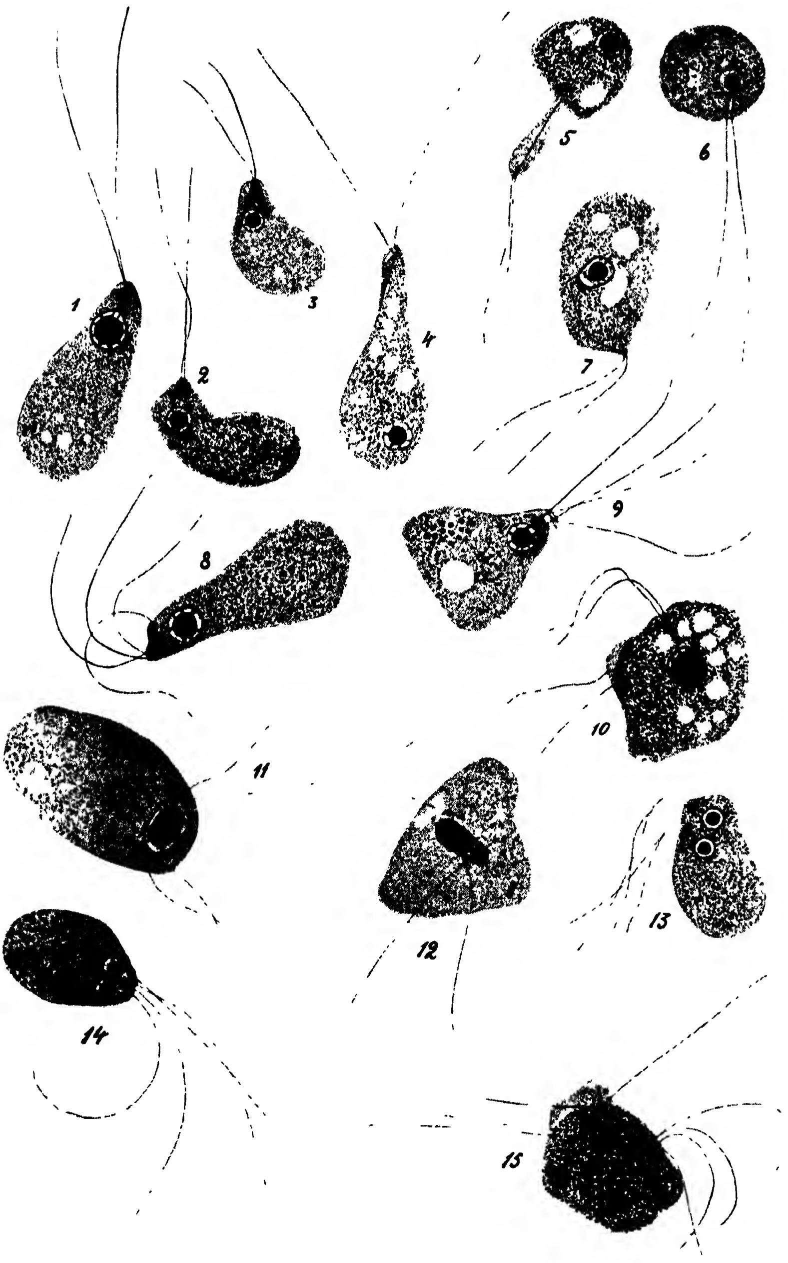


FIG. 120.—FLAGELLATE FORMS OF *Dimastigamœba gruberi* DEVELOPED A FEW HOURS AFTER PLACING THE AMŒBÆ IN TAP WATER (× *ca.* 1,400). (ORIGINAL.)

[*For description see opposite page.*

commenced. This would be in agreement with what is known to occur in other flagellates, in which the first stage of division of the organism is division of the blepharoplasts. These appearances suggest that the blepharoplasts are present in the cytoplasm even during the amœboid phase of the organism. They are so minute that, unless their connection with the flagella can be detected, it is impossible to distinguish them from other granules which occur in the cytoplasm. It seems probable that in the amœboid phase they are adjacent to, or actually upon, the nuclear membrane, and that when flagella are to be formed they move towards the surface of the body, retaining in many cases a connection with the nuclear membrane. There seems to be no real evidence that they are derived from the karyosome of the nucleus. Exceptionally only one flagellum is developed by uninucleated amœbæ, and three by binucleated or even uninucleated amœbæ.

The amœbæ themselves, judging from cultures commenced from a single individual, vary in size from about 5 to 20 microns (Figs. 61 and 121). They are fairly actively motile, and usually form blunt pseudopodia, but sometimes fine hair-like or radiating pseudopodia are produced, giving the organism the appearance of a Heliozoan. The nucleus contains a large central karyosome connected with the nuclear membrane by radiating septa which traverse the clear space. On the inner surface of the nuclear membrane, and just internal to it, is a layer of granules of chromatin. The amœbæ are difficult to recognize from others of the genus *Hartmannella*, unless the stages of nuclear division, the cysts, or the production of flagellates can be observed. The nuclear division has been described above (p. 103). The cysts are spherical structures, which in uninucleated forms vary in size from 5 to 12 microns. Their most characteristic feature is the presence of a number of pores in the cyst wall, which is composed of a double membrane (Fig. 121). On agar plates not only do the uninucleated amœbæ encyst, but also the multinucleated forms, which produce correspondingly larger cysts with an increased number of pores. Cysts from 12 to 18 microns in diameter have usually two nuclei, while larger ones have more. Thus a cyst 31 microns in diameter had three nuclei and twenty to thirty pores, while another was 21 microns in diameter, had six nuclei, and fifteen to twenty pores. The cytoplasm of encysted forms contains a number of conspicuous refractile bodies which may be larger than the nuclei. They stain black with iron hæmatoxylin, and are probably of a volutin nature.

1-7. Various forms of biflagellate type.
8-10. Forms with single nucleus, four flagella, and two pairs of blepharoplasts.
11-12. Forms with dividing nuclei, four flagella, and two pairs of blepharoplasts.
13-14. Forms with two nuclei, four flagella, and two pairs of blepharoplasts.
15. Form with four nuclei, eight flagella, and corresponding blepharoplasts.

Whitmore (1911*b*), working in the Philippines with cultures of amœbæ isolated from water on agar plates, noted that a certain amœba developed a stage with three flagella. His figures also show forms with two and four flagella. He gave it the name *Trimastigamœba philippinensis*. The amœboid phase was 16 to 18 microns in diameter, while the cyst was oval, and measured 13 to 14 microns by 8 to 12. In the flagellate phase, with flagella slightly shorter than the body, the organism was elongated, and measured 16 to 22 microns by 6·5 to 8 microns. As *Dimastigamœba gruberi* may sometimes develop three, four, or more flagella, it is clear

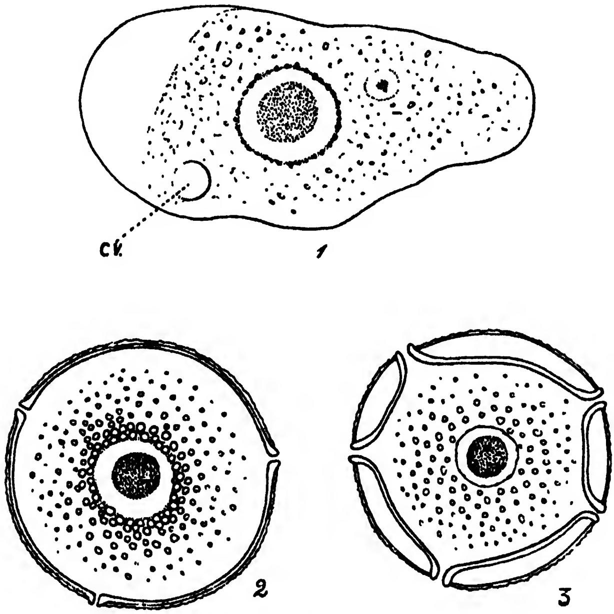


FIG. 121.—AMŒBOID PHASE OF *Dimastigamœba gruberi* OFF AGAR PLATE (×3,000). (ORIGINAL.)

1. Amœba showing nucleus with large central karyosome and peripheral granules and contractile vacuole (*cv.*).
2. Encysted form showing double membrane of cyst wall and three pores.
3. Encysted form in which inner membrane has separated from the outer membrane except at the pores.

that Whitmore was actually observing this species, his name *Trimastigamœba* becoming a synonym. From the work of Bunting (1922) it appears that flagellates of the genus *Tetramitus* may also have an amœboid phase (see p. 310).

4. *Family:* RHIZOMASTIGIDÆ Calkins, 1902.

This family includes certain free-living amœbæ, and possibly some parasitic forms which possess a single flagellum. They are usually classed

with the Rhizopoda, and not with the Mastigophora, because they live mostly as amœbæ and crawl about by means of pseudopodia, instead of swimming by means of their flagella. Unlike the members of the family Dimastigamœbidæ, they retain the flagellum throughout the amœboid phase. There are three genera. The genus *Mastigamœba* includes large amœbæ with a flagellum as long as, or longer than, the body. The axoneme of the flagellum arises from the nuclear membrane. The genus *Mastigella* includes similar forms, in which the flagellum is unconnected with the nucleus. The genus *Mastigina* comprises amœbæ which have a short flagellum, the axoneme of which arises from the nuclear membrane. The majority of these forms are free living, and for one of these Goldschmidt (1907) described a complicated life-cycle, which, however, has not received confirmation. A few parasitic forms have been described. Frenzel (1892) described as *Tricholimax hylæ* a flagellated amœba from the intestine of tadpoles of the genus *Hyla* in the Argentine (Fig. 73). Goldschmidt (1907) placed it in the genus *Mastigina.* Collin (1913) studied this organism, *Mastigina hylæ*, in tadpoles of newts and *Bufo calamita* in Europe. Spherical cysts, 25 to 28 microns in diameter, were produced, and these contained two or four nuclei. What was possibly the same organism was seen by Hoare, working in the writer's laboratory, in the intestine of *Triton vulgaris* in England. Becker (1925) has seen it in tadpoles of *Rana clamata* and *R. catesbiana* in America. He notes that the single short inactive flagellum arises from a blepharoplast situated on the nuclear membrane, and at one end of a cap-like structure which partially covers the nucleus. From the whole surface of the cap radiating fibres pass into the cytoplasm. From the blepharoplast a deeply staining curved rod passes into the cytoplasm. It is homologized with the basal fibre (rhizostyle) of the membrane of *Trichomonas.* Becker sees in the structure of this organism a ground plan of the morphology of such flagellates as *Trichomonas*, *Chilomastix*, and even *Giardia.*

Another parasitic form is that described by Liebetanz (1910) as *Mastigamœba bovis* from the rumen of cattle. It measures about 25 microns in longest diameter, and is provided with a flagellum about twice as long as the body. The cytoplasm is differentiated into a well-marked ectoplasm and endoplasm, and there is a large central nucleus.

II. *CLASS:* MASTIGOPHORA Diesing, 1865.

CLASSIFICATION.

CLASS: **MASTIGOPHORA**

SUB-CLASS: **Phytomastigina**

Order: CHRYSOMONADIDA
,, CRYPTOMONADIDA
,, DINOFLAGELLATA
,, EUGLENOIDIDA
,, PHYTOMONADIDA

SUB-CLASS: **Zoomastigina**

Monozoic Forms

Order: PROTOMONADIDA

Sub-Order: **Eumonadea**

Family: MONADIDÆ
,, TRYPANOSOMIDÆ
,, BODONIDÆ
,, PROWAZEKELLIDÆ
,, EMBADOMONADIDÆ
Family: CHILOMASTIGIDÆ
,, CERCOMONADIDÆ
,, CRYPTOBIIDÆ
,, TRICHOMONADIDÆ
,, DINENYMPHIDÆ

Sub-Order: **Craspedomonadea**

Order: HYPERMASTIGIDA
,, CYSTOFLAGELLATA

Diplozoic Forms

Order: DIPLOMONADIDA

Genus: **Hexamita**
,, **Giardia**
,, **Trepomonas**

Polyzoic Forms

Order: POLYMONADIDA

Family: CALONYMPHIDÆ

The Protozoa which are included in the class Mastigophora (=Flagellata Cohn, 1853) are commonly known as flagellates, and comprise a very varied assemblage of organisms which have one feature in common—namely, the possession of one or more flagella. In the case of Protozoa belonging to other classes, flagella may be temporarily present at certain stages of development, as, for instance, in the case of the microgametes of coccidia; but in the Mastigophora the flagella are present during the greater part of the life of the individual, and occur in the active, fully-grown, motile stage of the organisms. The majority of the Mastigophora are free-swimming creatures which move about in liquid media by the lashings of their flagella. Some of them resemble amœbæ more than flagellates, for, in addition to swimming, they may crawl over surfaces by means of pseudopodia. Others, again, secrete filaments or stalks, by means of which they are attached to objects. In some cases there is developed at the end of the filament a cup-like receptacle (lorica) in which the flagellate is lodged (Fig. 18). In other cases, groups of flagellates are held together by a common gelatinous matrix, the whole colony moving about as a single unit as a result of the joint action of the flagella of the several individual flagellates.

In what may be regarded as the most primitive forms the body consists of a portion of cytoplasm showing no differentiation into ectoplasm

or endoplasm. Superficially, it is covered by an exceedingly delicate membrane or periplast, which does not prevent amœboid movements, formation of pseudopodia, or the ingestion of food particles at any point of the body surface. There is a single nucleus, while one or more basal granules or blepharoplasts, which lie upon the nuclear membrane or free in the cytoplasm, are also present. From each blepharoplast there arises an axoneme, which may be traced to the surface of the body, and thence into a flagellum, which consists of the axoneme covered by a sheath formed by the periplast. In the non-parasitic forms contractile vacuoles are present. Flagellates of this relatively simple type are usually free-swimming organisms, but some of them are able to attach themselves temporarily to objects by a process like a pseudopodium or, with or without losing their flagella, to crawl about like amœbæ for a time. The filaments and cup-like receptacles mentioned above are formed as secretions from the surface of the body, as also is the gelatinous matrix which binds together the colonial forms. These structures are not actually parts of the organism, and are not to be regarded as modifications of the superficial layer of the cytoplasm. The more highly developed flagellates may be considered to have arisen from the simpler forms by changes in the periplast or by the development of internal structures. With a thicker periplast there is still the possibility of change in body form, though this is limited, while the power of ingesting food at any part of the body surface is lost. A definite cytostome is developed, usually near the origin of the flagella. With further development of the periplast, the body becomes rigid and a definite body shape is acquired. In many cases this thickened rigid periplast is of a high degree of complexity, and may be elaborately marked. The flagella, when more than one are present, usually arise near together at the anterior end of the body. Sometimes, however, they are spread over a wider area. In certain forms some of the flagella arise from the anterior end of the body and one or two from the posterior end. In the case of the latter the axonemes may pass directly backwards through the cytoplasm from their respective blepharoplasts (*Giardia*, *Hexamita*), or they may pass forwards through the cytoplasm to the anterior end of the body and, turning backwards, pass over the surface of the body to enter the flagella when they reach the posterior end. When an axoneme passes over the surface of the body, the periplast may be raised into a ridge or membrane (undulating membrane), along the edge of which the axoneme passes (*Trichomonas*, *Trypanosoma*). In certain flagellates the periplast at the anterior end of the body becomes raised into a collar or cuff, which surrounds the flagella (Choanoflagellata, or collared flagellates).

In addition to the complexities in organization which are the result of

elaborations of the superficial layer of the body, there occur others which arise from the formation of internal structures. In *Trichomonas* there is developed an organ called the axostyle, which is traceable through the body from the region of the blepharoplasts to the posterior end, through which it projects as a pointed rod. It is supposed by some to be a modified axoneme (see p. 42). Similarly, in this flagellate, which possesses an undulating membrane, a stiff fibre is developed along the base of the membrane. In *Chilomastix* the edge of the cytostomal groove is rendered rigid by two fibres which pass along its margins. The blepharoplast may be a simple granule in which the axoneme originates, or associated with it there may be another body of variable size and shape—the parabasal. The parabasal and the blepharoplast may be intimately connected, as in the trypanosomes and allied flagellates, to form a compound organ—the kinetoplast.

The majority of Mastigophora are uninucleated, and possess one or, at most, a small number of flagella with a corresponding number of blepharoplasts, which are usually closely grouped together, so that the individual blepharoplasts may be difficult to detect. The order Hypermastigida, however, includes flagellates which, though uninucleated, possess a large number of flagella and blepharoplasts.

The members of the order Diplomonadida (*Giardia* and *Hexamita*) have two nuclei and eight flagella and blepharoplasts, while the members of the order Polymonadida are multinucleate, and have a large number of flagella and blepharoplasts.

Reproduction amongst the Mastigophora is usually by binary fission, the division being a longitudinal one, which commences as a rule at the flagellated end of the organism after the blepharoplast and nucleus have divided. This division may take place in the free-swimming condition, or after the flagellate has lost its flagella and become an amœboid or rounded form, or in some cases after encystment has taken place. Cyst formation as a means of protection against desiccation commonly occurs.

COPROZOIC MASTIGOPHORA.

As in the case of free-living amœbæ, the encysted forms of many free-living flagellates are able to withstand the action of the digestive fluids of an animal's intestine. They pass unchanged through the intestine, and liberate the flagellates in the fæces. There are thus coprozoic flagellates as there are coprozoic amœbæ. Some flagellates which live in stagnant water and infusions are able to live in the intestine, especially of cold-blooded animals. It is possible that the *Hexamita* of the frog's intestine is identical with a similar form which lives in water. Berliner

(1909) noted that *Copromonas major* occurred coprozoically in lizard's fæces, and that occasionally it occurred in the unencysted stage in the lizard's intestine. Some flagellates which are more truly parasitic, such as *Trichomonas*, are not only readily culturable in artificial media, but may survive for long periods in fæces outside the body, while others, such as *Giardia*, quickly die after leaving the body. Other forms, such as *Bodo* and *Cercomonas*, rarely if ever occur in the intestine in any but the encysted stages, but they are the commonest forms to develop coprozoically in stale fæces.

INVASION OF BLOOD-STREAM BY INTESTINAL MASTIGOPHORA.

Between the forms which are more specially adapted to life in the intestine, like *Giardia* and *Trichomonas*, and the true parasitic flagellates belonging to the Trypanosomidæ and Cryptobiidæ various gradations occur. Several observers have found that intestinal flagellates may occasionally invade the blood-stream. Danilewsky (1889) noted that the intestinal *Hexamita* sometimes invaded the blood vessels of the edible frog and tortoise. Labbé (1894), and more recently Ponselle (1919), made a similar observation in the case of the frog. The latter was able to produce a blood infection of *Rana temporaria* by inoculating blood containing *Hexamita* from an infected edible frog. Labbé (1894) also stated that he had seen a *Bodo* and *Hexamita* in the blood of a lizard (*Lacerta* sp.), while *Hexamita* has been seen in the blood of the toad (*Bufo calamita*) in large numbers by Lavier and Galliard (1925). Lanfranchi (1908) saw *Trichomonas* in the blood of a pigeon. Gonder (1910*b*) observed *Giardia* in the blood of a falcon (*Elamus cœruleus*) which had been shot. It is possible that in this case the blood was contaminated from a wounded intestine. Martoglio (1917) saw a *Tetratrichomonas* in the blood of a fowl, while Chatton (1918*a*) observed a *Eutrichomastix* in the blood of the gecko (*Tarentola mauritanica*). Reichenow (1918) observed the same flagellate in the blood of *Lacerta muralis* and *L. viridis*. He noted that the mites which fed on the lizards also became infected, and that young lizards were able to acquire an intestinal infection by eating infected mites. During the examination of the blood of animals which had died in the Zoological Gardens in London, Plimmer (1912*a*) on several occasions found intestinal flagellates in the blood-films. He observed *Hexamita* in the blood of tortoises (*Cyclemys trifasciata*, *Cistudo carolina*, *Testudo angulata*), and *Trichomonas* in the blood of snakes (*Coluber leopardinus*, *Naia tripudians*, *Heterodon simus*, *Python sebæ*). It is possible that in some of these cases the flagellates appeared in the blood-films as a result of damage to the intestine at the *post-mortem* examination. Sangiorgi (1922) states that he observed a *Trichomonas*

in the heart blood of a dead mouse, and considered the flagellate had passed from the intestine into the blood-stream; while Knowles, Napier, and Das Gupta (1923) saw the flagellate in the liver and spleen of a rat during the course of kala-azar investigations. In the case of human beings, the writer (1920) found that the whole mucosa of the large intestine of a case was invaded by *Trichomonas*, while Pentimalli (1923) saw the same organism in the blood of a patient on two occasions at ten hours' interval. Several instances of the occurrence of flagellates of the leptomonas type in the blood and intestine of lizards are mentioned below. In the majority of these cases the invasion of the blood by intestinal flagellates was discovered *post-mortem*, so that it is not improbable that it had occurred either after or shortly before the death of the animal. In other cases the animals were evidently ill and in such a condition that the natural resistance to such invasion may have been absent. The fact, however, that such an invasion may take place is some indication of the possibility of intestinal flagellates acquiring the habits of blood parasites.

FLAGELLATES WHICH MAY CONTAMINATE BLOOD AND ORGAN SMEARS.

In this place may be mentioned a number of flagellates which have been encountered in smears made from the blood and organs of various animals. These forms have been regarded as parasites, but this is more than doubtful, for a source of error which arises from time to time in blood work has not been excluded. The distilled water used in laboratories for Romanowsky staining may become contaminated with free-living flagellates in the bottle or even in the pipette used in the manipulations. When the water is added to the film, either for the dehæmoglobinizing of a thick film or for the dilution of the alcoholic stain on the slide, the flagellates adhere to the film, and are found as blue-staining bodies with red nuclei and one or more flagella according to the nature of the flagellate in the water. The assumption that the flagellates occur in the blood is easily made if their possible source is not recognized. Henry (1917) drew attention to this fallacy in connection with Dymond's supposed hæmogregarine of trench fever. The writer has encountered this fallacy on several occasions. In one case in particular he was asked to look at a remarkable flagellate in a malarial blood-film. The organism appeared with a blue vacuolated cytoplasm with red nucleus and flagellum. Red-staining bacteria were also present. The possible origin of these was recognized, and an examination of the distilled water which had been used for diluting the Leishman stain revealed their presence.

Franchini (1913) in Italy described a flagellate from the blood and liver of a patient who had been in Brazil. The organism was also examined and

reported upon by Brumpt (1913*b*). In the films it occurred as blue-staining cytoplasmic bodies, sometimes pigmented and with one or two red-staining masses. In two cases short flagella were noted. Furthermore, encapsuled forms described as cysts were found. Brumpt noted that the films contained the structures described by Franchini, as well as many bacteria, some of which were actually within the cytoplasm of the flagellates. The organism was placed in a new genus by Franchini as *Hœmocystozoon braziliense.* The presence of encysted forms and the occurrence of bacteria within the flagellates, as well as in other parts of the films, are quite inconsistent with the assumption that the flagellate originated from the

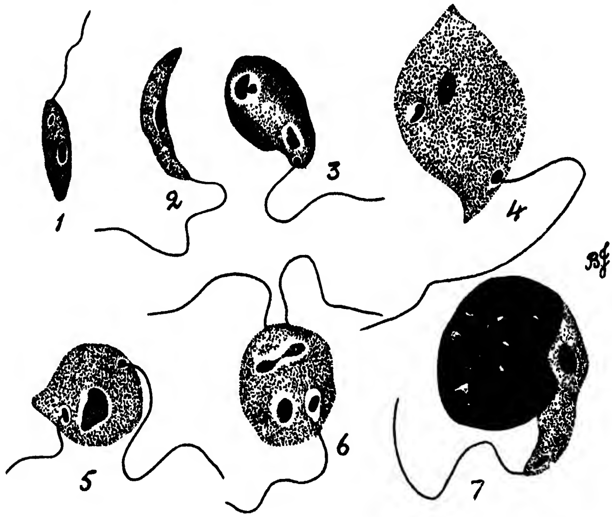


FIG. 122.—*Trypanopsis malignus*, AS SEEN IN DRIED SMEARS OF THE LIVER STAINED BY ROMANOWSKY STAIN (× *ca.* 4,000). (AFTER LEGER, M., 1920.)

One form appears to be ingested by a leucocyte, but the association is probably accidental. Similar forms were found in blood-films.

blood. The use of a distilled water infected with flagellates and bacteria could easily give rise to the appearances described, and this seems the probable explanation of their origin. Franchini attempted culture from the blood in N.N.N. medium, and no growth was obtained, a further confirmation of the view expressed here of their extraneous origin.

In the same category probably must be placed the flagellates described by Leger, M. (1920), from a fatal case of pyrexia in a human being in French Guiana. As figured by Leger, the organism appears as elongate flagellates with single flagellum, rounded forms with one to three flagella, and rounded forms without flagella (Fig. 122). Two chromatin masses were present, and the flagellum arose from one of them. In some of the rounded, non-

flagellate forms numerous chromatin bodies occurred. The organism was never plentiful in the films. It was named *Trypanopsis malignus* by its discoverer. The figures resemble very closely the forms which appear in films as described above, and the writer feels that the possibility of the flagellate having arisen from a slightly contaminated distilled water was not excluded.

Another fallacy which may occur is the result of contamination of exposed blood-films or smears by house-flies, which are very commonly infected with *Herpetomonas muscarum.* The flagellates are frequently passed in large numbers in the fæces of flies, and such fæces deposited on a film may be smeared over it by the fly itself or in some other way. When stained, the presence of flagellates in the film will be liable to cause confusion.

When an animal which has died is opened for examination, smears made from the liver, spleen, or other organs are very readily contaminated with the intestinal contents if the intestine has been opened even very slightly. Yeasts or even flagellates may thus contaminate the smears and lead to a wrong diagnosis. In practically all these cases it will be found that, in addition to the flagellates, the films contain a varied assemblage of bacteria, the presence of which should always give rise to suspicion. It is a common practice to open up animals which have been shot with the object of making films from the heart-blood and organs. A slight wounding of the intestine has often led to the passage of intestinal contents into the peritoneal cavity, and consequent contamination of blood-films.

DIVISION OF MASTIGOPHORA INTO SUB-CLASSES AND ORDERS.

Certain Mastigophora resemble plants in that they are provided with chromatophores containing chlorophyll, by means of which they lead a holophytic existence. They may secrete capsules composed of cellulose, while many of them possess red pigmented stigmata. These forms, which are very closely allied to the unicellular algæ, have been placed by Doflein (1916) in the sub-class **Phytomastigina,** to distinguish them from the **Zoomastigina,** which includes the flagellates which have a holozoic method of nutrition, and are evidently animal in nature. The latter ingest solid food at all parts of the body surface like amœbæ by means of pseudopodia or through a special opening, the cytostome, or they absorb by osmosis only preformed proteid matter in solution.

The members of the sub-class Phytomastigina are mostly free-living organisms which in many cases are closely related to the algæ. Many of them possess chlorophyll and have a holophytic mode of life. Reproduction is by binary fission, while syngamy, which is either isogamous or anisogamous, commonly occurs. Certain Euglenoidida are parasitic in

the intestine of tadpoles, while members of the genus *Copromonas* are commonly found in stale fæces. Following Doflein (1916) the sub-class **Phytomastigina** is divided into five orders:

1. *Order:* CHRYSOMONADIDA.

Simple forms of small size which possess chromatophores mostly coloured with a brown pigment. There are one or two flagella. Cysts

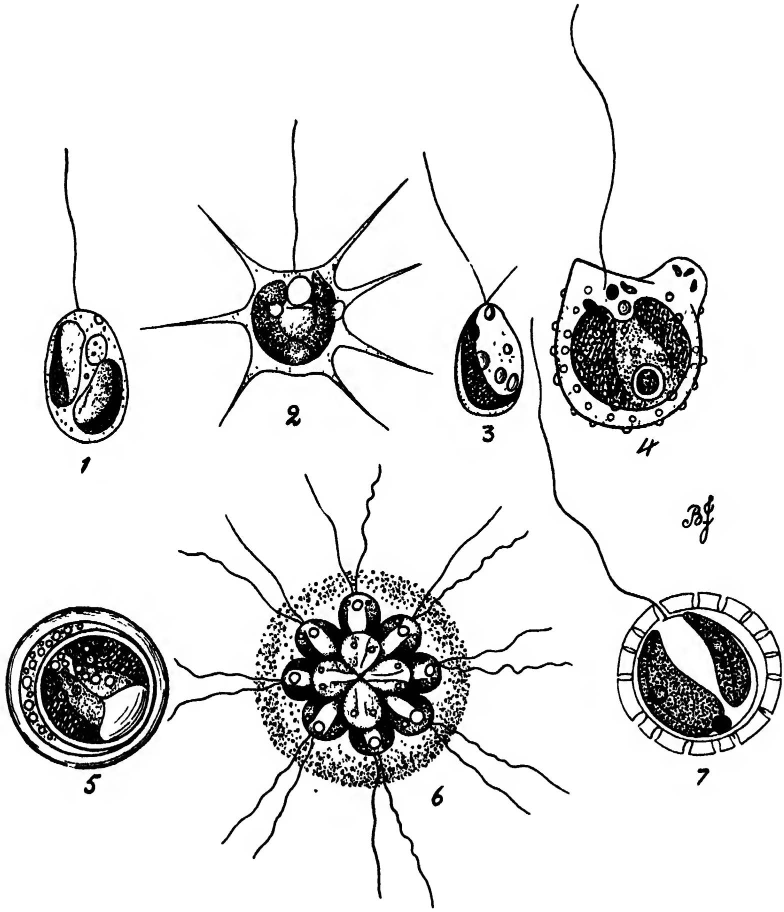


FIG. 123.—VARIOUS CHRYSOMONADIDA. (FROM OLTMANN, 1922, AFTER VARIOUS AUTHORS.)

1-2. *Chrysamœba radians* (× 1,000).
3. *Ochromonas simplex* (× *ca.* 400).
4. *Chromulina pascheri* (× *ca.* 1,500).
5. Cyst of *Chromulina flavicans* formed endogenously (× *ca.* 1,500).
6. *Syncrypta volvox*, a colonial form (× *ca.*650).
7. *Pontosphæra haeckeli* (× 1,600).

with siliceous walls are formed endogenously within the cytoplasm. The surface of the body may be limited by a rigid membrane, or such a structure

may be absent, the organism being capable of amœboid movements. Some forms develop cup-like loricæ in which they live. Numerous individuals may be held together by a gelatinous matrix to form colonies. The order (=Chrysomonadina Stein, 1878) includes *Chrysamœba*, *Ochromonas*, *Chromulina*, *Pontosphæra*, and other genera (Fig. 123).

2. *Order:* CRYPTOMONADIDA.

Small forms with two flagella and a thick, rigid periplast, which gives them a characteristic ovoid shape. The body is often flattened, while a longitudinal groove is frequently present on one surface. This asymmetry permits of a definite orientation. Chromatophores of varying colour are usually present. Included in the order (=Cryptomonadina Stein, 1878) are *Cryptomonas*, *Chilomonas* and other genera (Fig. 124).

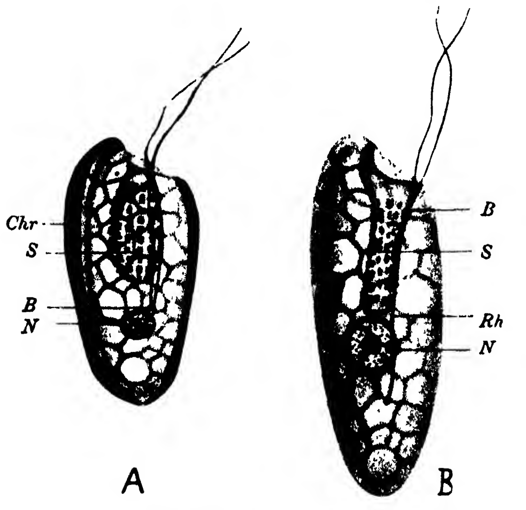


FIG. 124.—A, *Cryptomonas ovata*; B, *Chilomonas paramecium* (× 1,000). (AFTER DOFLEIN, 1916.)

Chr, Chromatophore; *B*, blepharoplast; *N*, nucleus; *S*, œsophagus; *Rh*, rhizoplast.

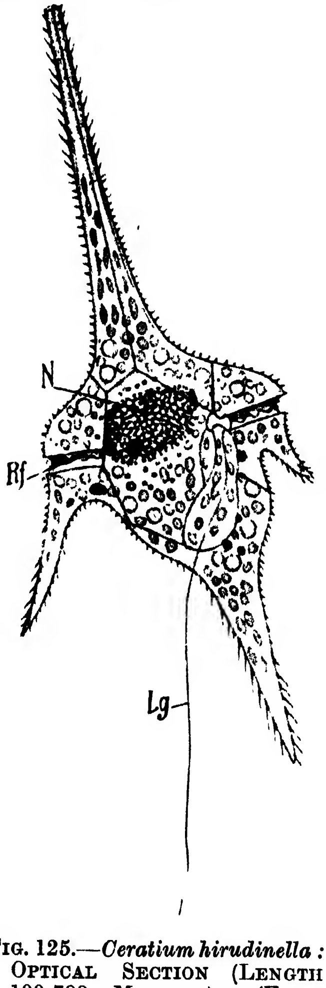


FIG. 125.—*Ceratium hirudinella*: OPTICAL SECTION (LENGTH 100-700 MICRONS). (FROM DOFLEIN, 1916, AFTER LAUTERBORN.)

N, Nucleus; *Rf*, equatorial groove with flagellum; *lg*, long free flagellum.

3. *Order:* DINOFLAGELLATA BÜTSCHLI, 1885.

These organisms, known also as Peridinians, which are mostly marine forms, have a thick, rigid covering to the body, which is variously shaped.

There are two flagella, one of which usually lies in a groove in the thick covering of the body. Chromatophores may or may not be present. There are a large number of genera, of which *Ceratium*, *Gymnodinium*, *Diplodinium*, and *Goniodoma* are representatives (Figs. 125, 126).

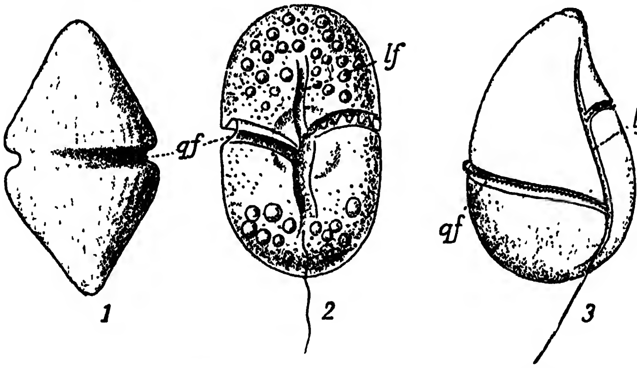


FIG. 126.—*Gymnodinium rhomboides* (1 AND 2), AND *G. spirale* (3) (×*ca.* 1,000). (FROM OLTMANN, 1922, AFTER SCHÜTT.)

qf, Equatorial groove; *lf*, longitudinal groove.

4. *Order:* EUGLENOIDIDA.

Large forms covered with a definite periplast often longitudinally marked. The shape of the body may be permanent or it may change according to the rigidity of the periplast. At the anterior end of the body is a depression, in which the flagellum arises. Sometimes there are two flagella. In some forms a cytostome leading to an œsophagus occurs in the anterior depression. There is a characteristic system of excretory vacuoles, consisting of a reservoir into which discharge one or more contractile vacuoles. A red pigment spot, the stigma, is often found at the anterior end of the body, while green chromatophores are frequently seen in the cytoplasm. The order (=Euglenoidina Bütschli, 1884) includes well-known genera such as *Euglena* (Figs. 6 and 128, B), *Astasia* (Fig. 127), and *Phacus* (Fig. 128, A), and the coprozoic *Copromonas* (Fig. 133).

5. *Order:* PHYTOMONADIDA.

These forms, which are often considered to be unicellular algæ, possess definite cellulose walls and are devoid of cytostome. There are usually two flagella, which emerge through a pore in the cell wall. Green chromatophores often occur, while some are coloured red by a pigment known as hæmatochrome. Red-pigmented stigmata are not infrequently present. Colonial grouping of a varying number of individuals is a common feature, while there may be a complicated life-history, in which syngamy is associated with the production of differentiated male and female gametes

as in *Volvox* (Fig. 129). Included in the order (=Phytomonadina Blochman, 1895), amongst other genera, are *Chlamydomonas* (Fig. 130); *Hæmatococcus* (Fig. 131), *Polytoma* (Fig. 42), *Parapolytoma* (Fig. 31), and colonial forms like *Gonium* (Fig. 132), *Pandorina*, and *Volvox*.

In the sub-class Zoomastigina are found numerous free-living forms as well as the various parasitic or saprophytic flagellates which occur in man

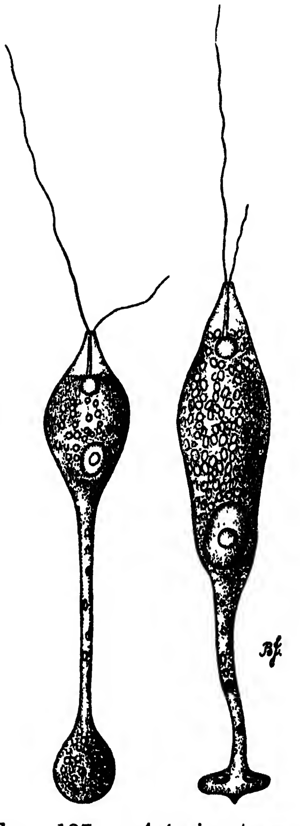


FIG. 127.—*Astasia tenax* (× 650). (AFTER STEIN, 1878.)

Two individuals, showing changes in form due to peristaltic waves of contraction; each possesses a nucleus, two flagella, and œsophagus, at base of which is a contractile vacuole.

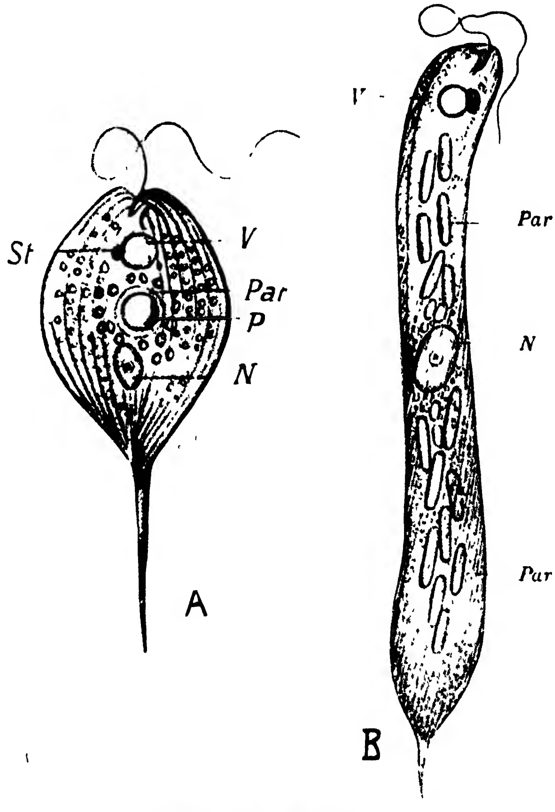


FIG. 128.—*Phacus longicaudus* (A) (× 650) AND *Euglena oxyuris* (B) (× 450). (FROM DOFLEIN, 1916, AFTER STEIN.)

Par, Paramylum; *P*, pyrenoid; *N*, nucleus; *V*, contractile vacuole; *st*, stigma.

and animals. The sub-class is usually divided into several orders as follows: Order Protomonadina, including simple forms with few flagella; the Polymastigina, more complex forms with several flagella and possibly other organs; the Hypermastigina, forms which are mostly parasitic in

white ants, and which have a very complex structure and large numbers of flagella; and the Cystoflagellata, marine flagellates of peculiar organiza-

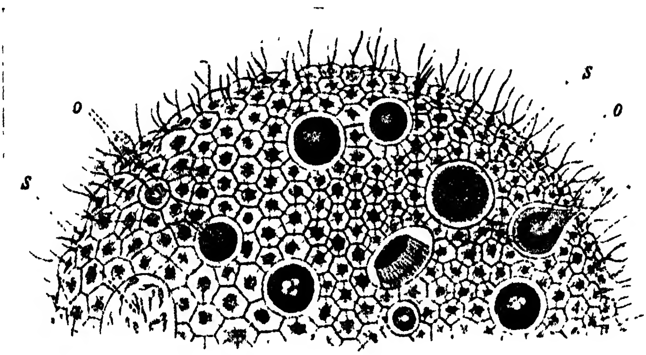


FIG. 129.—PORTION OF A SPHERICAL COLONY OF *Volvox globator*, IN WHICH SEXUALLY DIFFERENTIATED GAMETES HAVE DEVELOPED (× *ca.* 1,000). (FROM LANG, 1901, AFTER CIENKOWSKY AND BÜTSCHLI.)

S, Male gametes; O, female gametes.

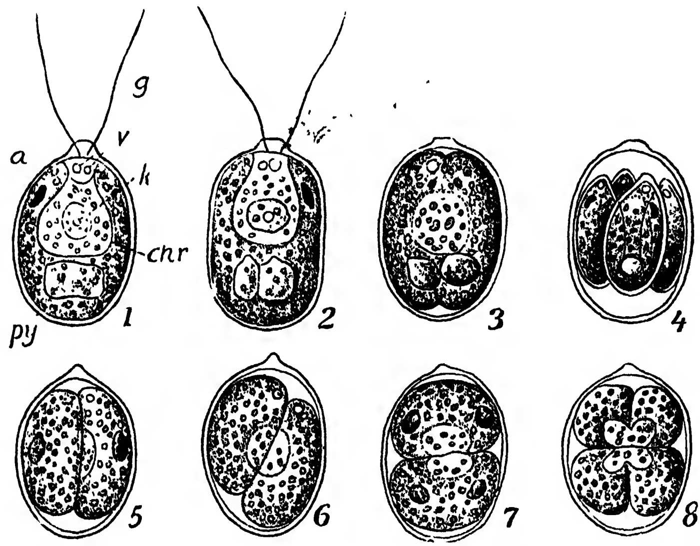


FIG. 130.—*Chlamydomonas angulosa* (1-4) AND *C. longistigma* (5-8), SHOWING METHOD OF MULTIPLICATION (× *ca.* 1,000). (FROM OLTMANN, 1922, AFTER DILL.)

a, Stigma; *chr*, chromatophores; *g*, flagella; *k*, nucleus; *py*, pyrenoids; *v*, contractile vacuole.

tion. Doflein separates from the Polymastigina, in the order Distomatina, certain flagellates (*Hexamita, Giardia*) which have a bilateral symmetry associated with the presence of two nuclei and two sets of

organs. The three last-named orders are fairly well defined, but there is more difficulty in connection with the Protomonadina and the Polymasti-

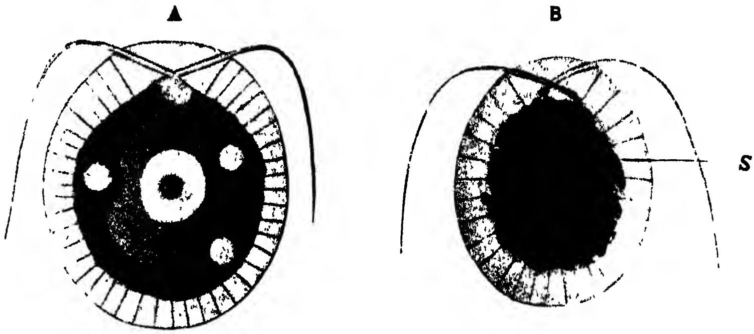


FIG. 131.—*Hæmatococcus pluvialis* (× *ca.* 2,500). (AFTER REICHENOW, 1910).

A. Individual from a culture in a special medium, giving rise to forms without hæmatochrome, The nucleus, three pyrenoids, and the stigma, as a dark rod near the right-hand margin. are clearly visible.

B. Usual form with structure obscured by hæmatochrome. S, stigma scarcely visible.

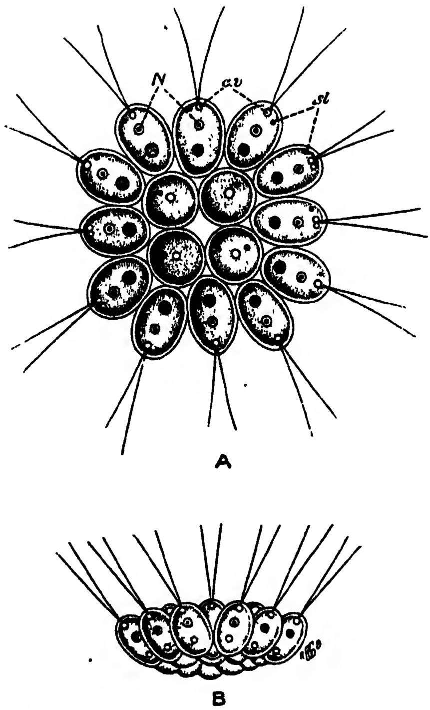


FIG. 132.—*Gonium pectorale*: COLONY OF SIXTEEN INDIVIDUALS, EACH WITH TWO FLAGELLA (× *ca.* 480). (FROM MINCHIN, 1912, AFTER STEIN.)

A, In surface view; *B*, in side view; *N*, nuclei; *c.v.*, contractile vacuole; *st*, stigmata.

gina. The two merge into one another, and certain forms which are usually placed in the one order might with equal justification be transferred to the other. It would seem better, therefore, to consider most of the flagellates usually included in these two orders as belonging to one order, Protomonadida, and to reserve an order, Polymonadida, for the flagellates belonging to the family Calonymphidæ, which includes parasitic forms possessing many nuclei and blepharoplasts from which arise a large number of flagella.

Hartmann and Chagas (1910*a*) divide their Protomonadina into two sub-orders—the Monozoa, including forms in which there is only a single nucleus and set of organs; and the Diplozoa, those which have a bilateral symmetry and double set of organs. It seems better, however, as Doflein has done, to separate the Diplozoic forms in another order entirely, for which the name Diplomonadida may be employed. The Zoomastigina can be considered from the point of view of the number of nuclei the adult forms possess, and this is perhaps the best basis for their primary subdivision. The majority of forms possess a single nucleus, and these can be regarded as Monozoic forms; others (*Giardia*) possess two nuclei, and are therefore Diplozoic; while others again (Calonymphidæ Grassi, 1911) have many nuclei, and are therefore Polyzoic (Figs. 291, 301).

The sub-class **Zoomastigina** may, therefore, be subdivided as follows:

A. Monozoic Forms.

There is a single nucleus and a varying number of flagella and blepharoplasts.

1. *Order:* PROTOMONADIDA.—The flagella are few in number (rarely more than six).

2. *Order:* HYPERMASTIGIDA.—The flagella are very numerous.

3. *Order:* CYSTOFLAGELLATA Haeckel, 1873.—The body is large and globular, and possesses a peculiar tentacle as well as a single flagellum.

B. Diplozoic Forms.

There are two nuclei, while the flagella, blepharoplasts, and other structures are similarly duplicated, giving rise to a bilateral symmetry.

4. *Order:* DIPLOMONADIDA.—With the characters of the Diplozoic forms.

C. Polyzoic Forms.

There are more than two nuclei and numerous flagella and blepharoplasts.

5. *Order:* POLYMONADIDA.—With the characters of the Polyzoic forms.

1. *SUB-CLASS:* **Phytomastigina** DOFLEIN, 1916.

The majority of flagellates belonging to this sub-class are free-living organisms. Certain Euglenoidida of the genus *Copromonas* commonly occur in stale fæces, while others are parasitic in the intestine of tadpoles.

Copromonas subtilis Dobell, 1908.—This organism, for which Dobell 1908*b*) established the genus, has an elongate body covered by a rigid

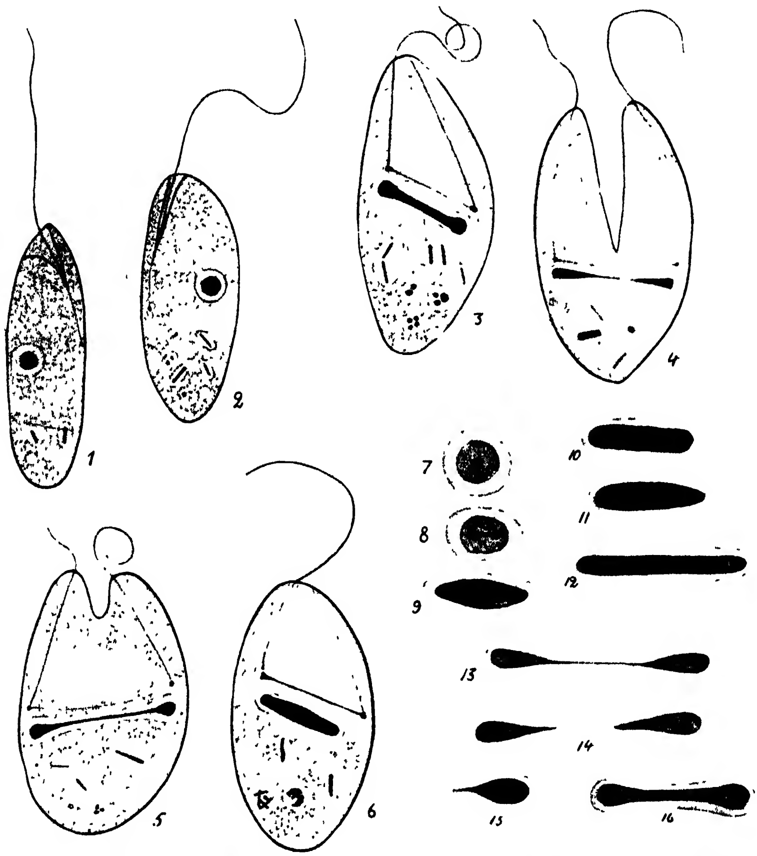


FIG. 133.—*Copromonas subtilis:* A COPROZOIC FLAGELLATE FROM FÆCES (1-6, × 2,600; 7-16, × 4,000). (ORIGINAL.)

1-2. Typical flagellates. 3-6. Stages in division.
7-15. Successive stages in division of nucleus. The decolorized karyosome appears to have a central granule which divides. The two halves remain connected by a fibre.
16. Connecting fibre of two halves of dividing central granule.

periplast. It is ovoid in outline and distinctly flattened, and possesses a cytostome leading to a long, narrow œsophagus. There is a single flagellum, which arises from the wall of the œsophagus. The nucleus is central in position, while a blepharoplast lies anterior to it. It is possible

that the form which was named *Copromonas subtilis* by Dobell is identical with *Scytomonas pusilla* Stein, 1878.

Copromonas subtilis was first described by Dobell (1908*b*) from the fæces of frogs and toads. Dobell and O'Connor (1921) report its occurrence once in human fæces, not as a parasite in the freshly passed stool, but as a coprozoic organism which had evidently developed from cysts after the stool had been passed. The writer has seen this flagellate in cultures of pig's fæces. It is an elongate organism with an average length of 15 microns (Fig. 133). Longer forms up to 20 microns and smaller ones of 4·5 microns also occur. The body is covered with a thick, rigid pellicle, so that there is little change of shape. The anterior end is somewhat pointed, and there is here a cytostome leading to an œsophagus which extends through half the length of the body, the posterior end of which is rounded. There is a single flagellum, which arises from a blepharoplast situated in the wall of the œsophagus near the nucleus. During forward progression the tapering flagellum projects as a rigid filament, the movements being confined to the distal third or half. According to Dobell, near the blepharoplast is a clear vesicle, the reservoir, into which the contents of a minute contractile vacuole are periodically discharged. The nucleus is centrally placed, and consists of a spherical membrane and a large central karyosome. Multiplication is by longitudinal division from before backwards, after division of the nucleus and blepharoplast. The flagellum is discarded, and after division of the blepharoplast two new flagella are developed as outgrowths from the two daughter blepharoplasts, which, during division, remain connected by a long fibre which lies transversely across the body and parallel to the spindle of the dividing nucleus. Syngamy occurs, as first described by Dobell (1908*b*). Two flagellates unite by their anterior ends, the union extending backwards till their two bodies are completely fused (Fig. 48). Each nucleus is described as undergoing a reduction of its chromatin, after which union takes place. During the conjugation one flagellum is withdrawn, so that the zygote has a single flagellum, by means of which it moves about actively. The zygote may commence dividing after leading a free existence for some time, or it may encyst. Encystment may also occur without conjugation. The cysts are ovoid or spherical structures with thin walls and clear contents. They measure 7 to 8 microns in length. Berliner (1909) gave the name *Copromonas major* to a form which he cultivated on agar plates from the fæces of lizards. Like the form cultivated from goat's fæces by Woodcock (1916), which he named *Copromonas ruminantium*, it is slightly larger than *C. subtilis*. Both these may be merely races of the smaller flagellate. Berliner stated that the flagellates were sometimes present in the free-swimming stage in the intestine of lizards.

In addition to the forms just considered, which are coprozoic in habit, certain Euglenoidida are definitely parasitic.

Tadpoles appear to be commonly infected with certain chlorophyll-bearing flagellates allied to the free-living *Euglena*. Alexeieff (1912*f*)

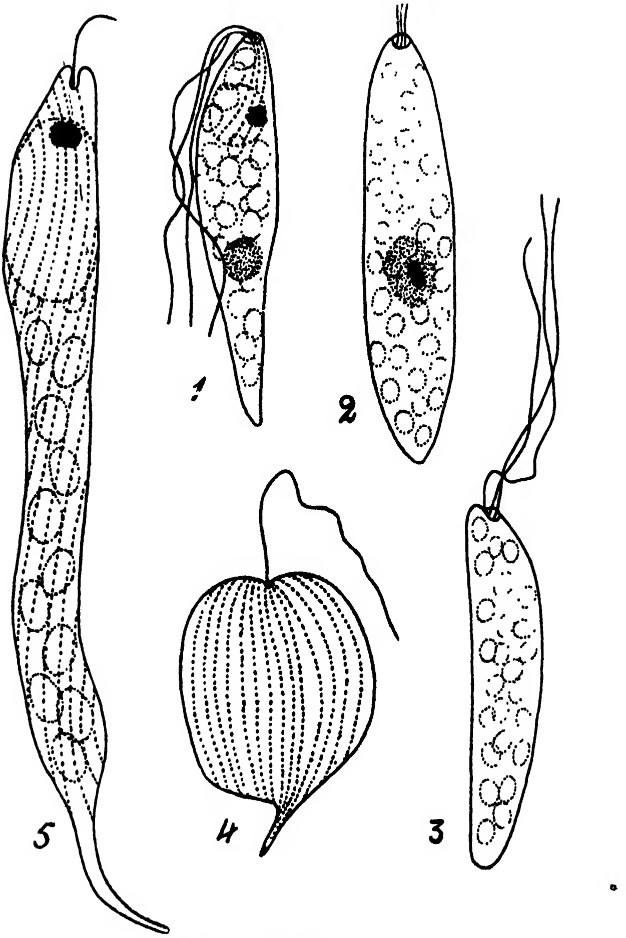


FIG. 134.—EUGLENOID FLAGELLATES FROM THE INTESTINE OF TADPOLES OF *Rana pipiens* AND OTHER SPECIES. (AFTER HEGNER, 1923.)

1-3. *Euglenamorpha hegneri* (× 1,600): (1) Living specimen showing three flagella, reservoir, stigma, chromatophores, and nucleus; (2) specimen fixed in Schaudinn's fluid and stained with iron hæmatoxylin; (3) specimen stained with iodine.
4. Living specimen of *Phacus* (× 1,600). 5. *Euglena spirogyra?* (× 780).

noted them in large numbers in the rectum, and states that Brumpt had made a similar observation. He placed the organism in the genus *Euglena* without giving it a specific name. He also observed a species of *Phacus* in the same host. He regarded the flagellates as accidentally present in

the intestine. He points out that the allied *Astasia captiva* described by Beauchamp (1911) from a turbellarian *Catenula lemnæ* was more truly parasitic, as it perished after removal from its host. Alexeieff (1912*f*) further records the presence of *Astasia mobilis* in a species of *Cyclops*. It occurred not only in the intestine, but also in the developing embryos in the egg sac, a fact which led Alexeieff to express the view that it might be transmitted hereditarily from host to host, and to restate Bütschli's theory that Sporozoa may have evolved from these or allied flagellates. Hegner (1923*c*) has given a description of Euglenoids studied by him in tadpoles in America (Fig. 134). One form had a single flagellum like the common free-living type *Euglena spirogyra*, while another possessed three flagella. To the latter Wenrich (1923) has given the name *Euglenamorpha hegneri*. It appears to be as truly parasitic as other Protozoa in the intestine. It does not survive when removed from its host for any length of time, but is readily passed from tadpole to tadpole by feeding. Hegner also noted the presence of a species of *Phacus*. The three types agreed with one another in the possession of green chromatophores and bright red stigmata. Another form discovered in tadpoles of *Leptodactylus ocellatus* of Brazil has been placed in a new genus *Hegneria* by Brumpt and Lavier (1924). The single species, *H. leptodactyli*, varies in length from 40 to 50 microns and has seven flagella. There is a large anterior vacuole across which the intracytoplasmic portions of the seven axonemes pass to end in seven blepharoplasts on the posterior wall of the vacuole.

2. *SUB-CLASS:* Zoomastigina Doflein, 1916.

A. Monozoic Forms.

1. *Order:* PROTOMONADIDA.

As already remarked, the flagellates included in this order (=Protomonadina Blochmann, 1895) are forms of relatively uncomplicated structure. They are monozoic, and possess a single nucleus and one or more flagella, each of which has an axoneme arising from a blepharoplast situated upon the nuclear membrane or separated from it. In the latter case there is often a complex structure, the kinetoplast, made up of a body called the parabasal and one or more blepharoplasts. The axoneme forms the central core of the flagellum. It arises from the blepharoplast, and usually takes a straight course to the surface of the body, whence it enters the flagellum. Sometimes, however, when the surface of the body is reached, it passes along the surface for some distance before entering the flagellum, and the line of attachment may be raised into a thin membrane. In some forms the cytoplasm at the anterior end of the body is raised

into a cylindrical collar or cuff around the base of the flagellum. The majority of flagellates belonging to the Protomonadida are free-swimming, but some of them develop attachment filaments, and it is in these forms that cup-like sheaths (loricæ) and collars commonly occur.

This order includes a large number of free, non-parasitic, and coprozoic forms, as well as certain parasites such as the trypanosomes and some of the intestinal flagellates of man and animals.

Many of the simpler Protomonadida are able to ingest solid food at any part of the body surface by means of pseudopodia, just as amœbæ do. These forms are sometimes known as the Pantostomatina.. Others, however, only ingest food near the base of the flagellum, where a permanent cytostome may or may not be present. In the case of the parasitic blood-inhabiting Trypanosomidæ and the Cryptobiidæ there is no cytostome, and nutrition is effected by the absorption of nutrient material from the blood in solution. In some saprophytic forms it is probable that both solid food is ingested as well as nutriment in a soluble form. The Protomonadida do not, as a rule, possess any accessory internal organs, but in some of them axostyles, parabasals, supporting filaments and other structures are developed.

The order PROTOMONADIDA may be subdivided into two sub-orders, the **Eumonadea**, which are free-swimming forms, and the **Craspedomonadea**, which possess attachment organs, and which may or may not have collars or loricæ.

(1). *Sub-Order:* **Eumonadea.**

The members of this sub-order are flagellates of relatively simple structure which have one or a small number of flagella. Each flagellum arises from a blepharoplast, which may be on the nuclear membrane or separate from it. When more than one flagellum is present, one may function as a trailing flagellum. Accessory structures such as axostyles are sometimes present. The following families may be recognized:

1. *Family:* MONADIDÆ Kent, 1880.—Flagellates of simple structure with one or more free flagella, the axonemes of which originate in blepharoplasts which are either upon the nuclear membrane or removed from it. When there is more than one flagellum, one may function as a trailing flagellum. The body, which is very metabolic, may or may not be provided with a cytostome.

2. *Family:* TRYPANOSOMIDÆ Doflein, 1901.—Flagellates which have a single flagellum and are parasitic in vertebrates, invertebrates, or plants. The body is usually elongate, and the axoneme of the flagellum in its course from the blepharoplast to the point of origin of the flagellum may, if the blepharoplast be near the nucleus or posterior to it, pass along the border

of an undulating membrane. There is no cytostome. The flagellates frequently assume a rounded leishmania form devoid of flagella.

3. *Family:* BODONIDÆ Doflein, 1901.—Flagellates which have two flagella, which arise near a laterally placed cytostome. One of the flagella is directed backwards as a trailing flagellum. A parabasal body is associated with the two blepharoplasts, which are separated from the nuclear membrane. The encysted forms are ovoid structures containing a single flagellate.

4. *Family:* PROWAZEKELLIDÆ Doflein, 1916.—Parasitic flagellates which have two flagella, one directed forwards and the other backwards as a trailing flagellum. The blepharoplasts are on the nuclear membrane. The cysts are spherical structures, which increase in size after they are first formed and produce within them a large number of daughter flagellates.

5. *Family:* EMBADOMONADIDÆ Alexeieff, 1917.—Flagellates with two flagella, one directed forwards and the other backwards or laterally through a large cytostome. The blepharoplasts, which lie near the nuclear membrane, are not associated with a parabasal. The cysts are ovoid or pear-shaped structures containing a single flagellate.

6. *Family:* CHILOMASTIGIDÆ.—Flagellates with four or more flagella, one of which lies in a large cytostomal groove, while the others are directed forwards. The blepharoplasts of the flagella are closely grouped together near the nucleus, and there is no parabasal. The margins of the cytostomal groove are supported by fibres. The cysts are ovoid or pear-shaped structures containing a single flagellate.

7. *Family:* CERCOMONADIDÆ Kent, 1880.—Flagellates which have one or more flagella, the axoneme of one of which passes backwards over the surface of the body, to which it is adherent, without development of an undulating membrane. The blepharoplasts are upon the nuclear membrane. The cysts are simple ovoid or spherical structures containing a single flagellate.

8. *Family:* CRYPTOBIIDÆ Poche, 1913.—Flagellates which have two flagella, one of which is directed forwards while the other passes backwards and is attached to the surface of the body, which may be raised into an undulating membrane. The two blepharoplasts are separate from the nucleus, and there is a parabasal associated with them. Cysts may or may not be produced.

9. *Family:* TRICHOMONADIDÆ.—Flagellates which have three or more flagella; one axoneme may pass backwards along the margin of an undulating membrane. The blepharoplasts form a group near the nucleus, and there may or may not be a parabasal. A pointed rod-like structure, the axostyle, passes through the cytoplasm from the anterior to the posterior end of the body, through which it protrudes. The cysts are ovoid or spherical, and contain a single flagellate.

10. *Family:* DINENYMPHIDÆ Grassi, 1911.—Flagellates which have several flagella, the axonemes of which are directed backwards and attached to the borders of a series of undulating membranes. There is an axostyle, as in the Trichomonadidæ.

(2). *Sub-Order:* **Craspedomonadea.**

The sub-order Craspedomonadea includes flagellates which are more or less permanently attached to objects (Figs. 16, 17, 18). The point of attachment is the posterior end of the body, and from this a filament may be secreted, at the end of which the flagellates wave about. In some cases the filament becomes a complex, tree-like system with a flagellate at the extremity of each branch. Each attached flagellate may develop around itself a gelatinous or chitinous cup-like sheath or lorica. The latter is formed both by attached flagellates, which have no filaments, as well as by those which possess them. Another modification undergone by some of these attached flagellates is the development of a cytoplasmic cylindrical collar or cuff with overlapping margins round the base of the flagellum at the anterior end of the body. The collared forms may or may not have loricæ as well. The Craspedomonadea are not parasitic forms, and they often appear in fluids containing decomposing vegetable matter such as hay infusion. They need not be considered any further here.

SYSTEMATIC DESCRIPTION OF THE GENERA AND SPECIES IN THE FAMILIES OF THE SUB-ORDER EUMONADEA.

The flagellates in this sub-order are unattached, free-swimming forms, some of which are parasitic, though the majority are not. They include types with a single flagellum and very simple structure, and a series of transition forms leading to more complicated flagellates with at least six flagella.

1. *Family:* MONADIDÆ Kent, 1880.

The flagellates belonging to this family include the simplest of the Mastigophora. They possess one or more flagella, the axonemes of which take origin in blepharoplasts which are situated either upon the nuclear membrane or separate from it. When there is more than one flagellum all may be directed forwards, or one may be differentiated as a trailing flagellum. A cytostome may or may not be present, while the body is often liable to marked amœboid changes of form. Apart from the nucleus, blepharoplasts, and axonemes there are no internal structures except the food vacuoles and the contractile vacuoles in the non-parasitic forms. The Monadidæ could be subdivided

into a number of sub-families according to the number of flagella present, but it seems unnecessary to give these names. The following groups can be recognized:

A. MONADIDÆ WITH ONE FLAGELLUM.

A number of minute flagellates which possess a single flagellum have been described from stagnant water and infusions. Some of these are exceedingly minute, and many of them may be the " swarm spores " of plants or other Protozoa. Undoubtedly, many of the forms which are included with the Phytomastigina might be classed with the Monadidæ, but for the purpose of this work they have been omitted.

Genus: **Oikomonas** Kent, 1880.

A typical member of this genus, as defined by Kent, has an ovoid or spherical body and a single flagellum, while the posterior end of the body

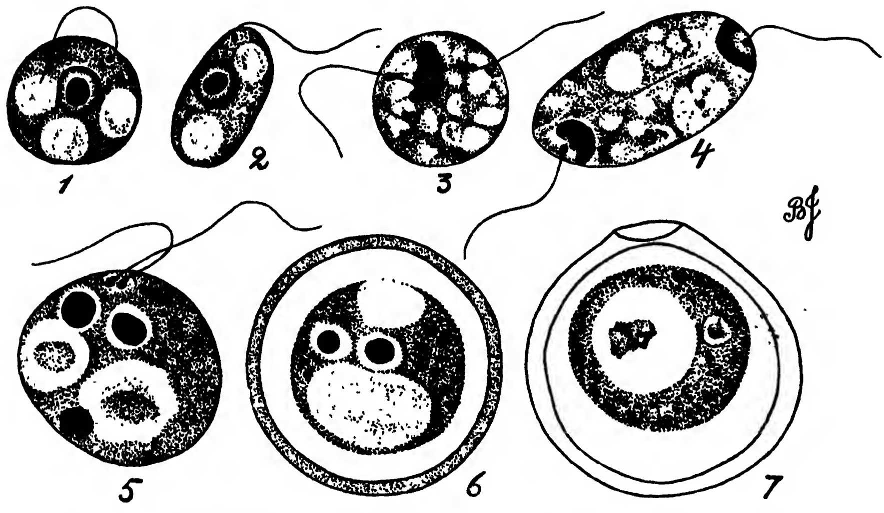


FIG. 135.—*Oikomonas termo* : FREE AND ENCYSTED FORMS (× *ca.* 2,000). (AFTER MARTIN, 1912.

1-2. Usual type. 3-4. Dividing forms. 5. Stage in conjugation. 6. Encysting zygote. 7. Mature cyst.

may form a pseudopodium by means of which temporary attachment to objects can be effected. Several species were described by Kent as occurring in stagnant water.

Oikomonas termo (Ehrenberg, 1838).—This flagellate, which is possibly identical with the flagellate described by Müller (1773) and Ehrenberg (1838) as *Monas termo*, and by Stein (1878) as *Cercomonas termo*, was studied by Martin (1912), who recovered it from soil (Fig. 135). The body,

when spherical, has a diameter of about 4·5 microns. It possesses a spherical nucleus with a large central karyosome. Near the surface of the anterior end of the body is a blepharoplast, from which arises a single flagellum which is as long as, or longer than, the body. Reproduction is by binary fission, and spherical resistant cysts are produced.

From the intestine of man and animals, several observers have described flagellates of this type. Liebetanz (1910), who examined the contents of the rumen of cattle, encountered several types of uniflagellate organism. A form which had an egg-shaped body and long flagellum springing from its narrow anterior end he placed in Kent's genus *Oikomonas*, while he created the genus *Sphæromonas* for a type with a spherical body, and the genus *Piromonas* for one with a pear-shaped body and a flagellum arising at a point a short distance behind its narrow anterior end. He further distinguished two species of *Oikomonas* (*O. communis* and *O. minima*), three of *Sphæromonas* (*S. communis*, *S. minima*, and *S. maxima*), and three of *Piromonas* (*P. communis*, *P. minima*, and *P. maxima*). The members of the genus *Oikomonas* varied in length from 4 to 11 microns, those of the genus *Sphæromonas* from 3 to 14 microns, and those of the genus *Piromonas* from 4 to 18 microns. It is clearly an error to establish these species on size alone. In fact, Braune (1913) united the species of *Sphæromonas* in the one species *S. communis*, while Fonseca (1916) believes that the genus *Piromonas* is identical with *Sphæromonas*, and that the difference in the shape of the body described by Liebetanz is only an indication of change in body form. He, nevertheless, records two species of *Sphæromonas* from cattle which differ from one another only very slightly (Fig. 136). He also records the finding of *S. communis* in the goat and guinea-pig (*Cavia porcellus*), as well as in cattle in Brazil.

FIG. 136.—*Sphæromonas communis* (1) AND *S. liebetanzi* (2) (× *ca.* 2,000). (AFTER FONSECA, 1916.)

It is undoubtedly fallacious to separate these uniflagellate organisms in different genera, as Liebetanz has done. It is not improbable that they all belong to the genus *Oikomonas*.

Under the name of *Oikomonas granulata* Yakimoff, Solowzoff, and Wassilewsky (1921) describe a small flagellate isolated by them by inoculating agar plates with the stools of two cases of diarrhœa in Petrograd. They distinguish the organism from the free-living form *O. termo* on account of the presence of certain granules in the cytoplasm. Yakimoff, Wassilewsky, Korniloff, and Zwietkoff (1921) state that they have isolated *O. termo* from the fæces of guinea-pigs and mice by employing the same technique. Another form isolated from guinea-pig fæces is described as *Sphæromonas rossica*, and one from rabbit fæces as *Piromonas rossica*. The description of these forms is most unsatisfactory, and there are no grounds whatever for the assumption made that the flagellates were actual parasites of man or animals. They were undoubtedly dealing with free-living forms which had passed through the intestine in the encysted state, or, what is more probable, with flagellates in water contaminating the vessels in which the samples of fæces were collected.

The writer has seen an organism of the *Oikomonas* type in the tortoise *Testudo calcarata*. The body is spherical or ovoid, and possesses a single long flagellum. When spherical, the body varies in diameter from 5 to 16 microns. There is a nucleus with large central karyosome, while the axoneme of the flagellum arises from a blepharoplast near the surface of the body.

The organisms discovered in human fæces by Kofoid and Swezy (1921*b*), which they regard as representing two species of *Craigia* (see p. 294), not improbably belong to the genus *Oikomonas*.

Blackhead of Turkeys.

This disease, which takes the form of an entero-hepatitis associated with black discoloration of the head, especially in young turkeys, may be considered here on account of its association with a flagellate infection. Theobald Smith (1895) described as *Amœba meleagris* certain structures which he found in the intestinal and liver lesions. Cole and Hadley (1910) believed that the amœbæ were really the schizogony stages of a coccidium which it was supposed had been acquired from sparrows. Theobald Smith and Smillie (1917), however, showed that the coccidium of the sparrow was an *Isospora*, while that of the turkey was an *Eimeria*. Hadley and Amison (1911) came to the conclusion that the lesions were not due to a coccidium, but to *Trichomonas* which had invaded the tissues and become mostly aflagellate amœboid bodies. Jowett (1911*a*), working in South Africa, came to the same conclusion. Hadley (1916, 1917), after further investigations, stated that he had actually seen flagella on some of the tissue forms, and was still further convinced of their

Trichomonas nature. Tyzzer (1919), however, refutes these statements, and returns to Theobald Smith's original view that the invading organism is actually an amœba, and that the disease is comparable to amœbic dysentery in man. Further investigations by Tyzzer (1920*a*) showed that the amœboid bodies which invaded the tissues exhibited peculiar jerky movement when seen alive, and this fact, combined with

FIG 137.—*Histomonas meleagris* FROM THE INTESTINE OF TURKEYS AFFECTED WITH BLACKHEAD (× 1,400). (AFTER TYZZER, 1919.)

a. Section of large intestine, showing parasites in mucosa. *b*. Dividing form. *c*-*d*. Forms showing nuclei and blepharoplasts, with attached fibres.

the presence of a blepharoplast from which axonemes appeared to pass to the surface of the body, and the formation of a fibril between daughter blepharoplasts when division occurs, strongly suggested flagellate affinities (Fig. 137). Tyzzer, however, did not believe that the parasites were *Trichomonas* which had lost their flagella after invasion of the tissues. He regarded them as aberrant flagellates, for which he proposed the name *Histomonas meleagris*, recognizing in them the bodies which Theobald

Smith originally called *Amœba meleagris.* Tyzzer and Fabyan (1920), and Tyzzer, Fabyan, and Foot (1921) showed that the disease could be produced in young turkeys by the subcutaneous inoculation of diseased tissues. Local lesions followed by generalized infection in the form of nodules occurred. The same result occasionally followed the inoculation of pigeons, whereas chickens only developed a local skin lesion. It had been pointed out by Smith and Graybill (1920) that blackhead could be produced in turkeys by feeding them with ova of *Heterakis papillosa.* Tyzzer, Fabyan, and Foot (1921) confirmed these observations, but concluded that the helminth was not the actual cause of the disease, but that it was merely one of the causes of the condition favourable to invasion of the body by *Histomonas meleagris.* In support of the conclusion that this organism is not simply the common *Trichomonas* of the intestine which has invaded the tissues, Tyzzer and Fabyan (1920) point out that blackhead may occur in young birds, which appear on examination of the intestine to be quite free from flagellates, and that feeding newly hatched turkeys with infected tissues does not lead to the appearance of flagellates in the gut.

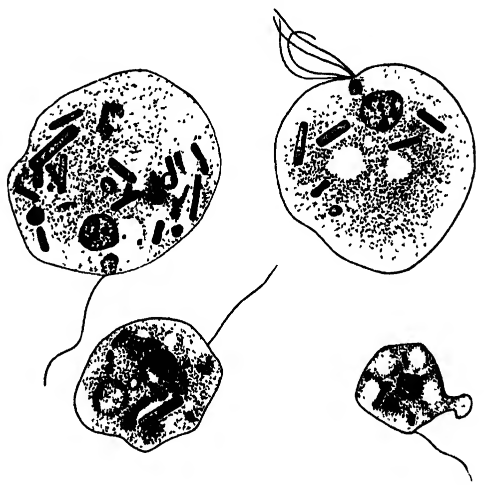


FIG. 138.—FLAGELLATES FROM THE FÆCES OF YOUNG CHICKENS INFECTED FROM TURKEYS SUFFERING FROM BLACKHEAD (× 2,000). (ORIGINAL FROM GIEMSA STAINED FILM PREPARED BY DRBOHLAV.)

Tyzzer (1924) and Drbohlav (1924) have now found that young chickens contract the disease when fed upon liver tissue of diseased turkeys. They are less susceptible to the disease than turkeys, and usually recover from the acute symptoms. When the acute symptoms abate, the intestine is found to harbour an organism which in many respects resembles an amœba, except that it is provided with one to four short flagella, which impart to the living organism a peculiar jerky movement, as noted by Tyzzer (1920) in the case of the tissue forms (Fig. 138). This infection occurs in chickens which have been carefully isolated and fed on sterile food, and the particular organism is the only one present apart from bacteria. Control chickens not fed upon liver tissue have no such infection. The recovered chickens with the intestinal infection are regarded as carriers. The organism, which, it is believed, is the same as the one which occurs in the

diseased tissues, can be cultivated from chicken fæces on egg medium, and the cultures fed to young chickens produce the same condition as that resulting from ingestion of liver material. It has not, however, been possible to obtain cultures directly from the tissues. From these observations it would appear that the organism named *H. meleagris* is actually a flagellate which has one to four flagella, the axonemes of which arise from a blepharoplast or group of blepharoplasts; that it lives in the intestine as a flagellate and is able to invade the tissues. There is no trace of axostyle, undulating membrane, or basal fibre, so that its relation to *Trichomonas* cannot be upheld. It seems possible that, as the majority of the flagellate forms have but one flagellum, this is the normal condition, and that the rarer forms with more than one flagellum are the result of precocious division of the blepharoplast. In many respects the organism resembles a member of the genus *Oikomonas.*

Genus: **Craigia** Calkins, 1913.

Calkins (1913) founded the genus *Craigia* for an organism said to be parasitic in the human intestine, and which was first described by Craig (1906) from the Philippines as *Paramœba hominis.* Barlow (1915) stated that he had discovered a similar but smaller organism in Honduras and named it *Craigia migrans.* He claimed to have seen over 150 cases of infection, and attributed to the presence of the organism the numerous symptoms, including fever, dysentery, and even abscess of the liver, from which his cases suffered. Such assertions it is manifestly impossible to accept. *C. hominis*, described by Craig, is said to live in the intestine of man, and to have both an amœboid and a flagellate stage. The amœboid form is described as resembling *E. coli*, and measuring in diameter 10 to 25 microns. It was said to form uninucleated cysts, from which, after further development, numbers of flagellates escape. The latter grow and attain a diameter of 10 to 20 microns. Each flagellate is depicted as consisting of a rounded body and a long tapering process, which, though described as a flagellum, certainly does not appear like one in the figures accompanying Craig's description. After many futile attempts to discover such an organism, the writer was very kindly given some preparations by its discoverer. In these, which, unfortunately, were poorly stained, the writer could find only typical free forms of *E. coli* and *Chilomastix mesnili*, a flagellate which was hitherto unrecorded from the Philippines, where the films were made. The three anterior flagella which this latter organism possesses were very difficult to detect on account of the imperfect staining. The posterior extremity of the organism, however, was drawn out in many cases into a tapering process which resembled the structures which were called flagella by the original discoverer of *C. hominis.* Both

the amœbæ and the flagellates were of the dimensions given by this observer for the corresponding stages of *C. hominis*. In these preparations no other Protozoa were present, so it seems probable that these had been regarded as *C. hominis*. When Barlow's description of *Craigia* appeared, the writer asked him for preparations, but was informed that none was available, and the films he had prepared were so poorly stained that he had not been able to recognize the nature of the organism, but that Craig, to whom he had sent the films, had been able to convince himself that *Craigia* was present. At the writer's request Dr. Newham, during a recent visit to Honduras, made films from a number of cases showing intestinal flagellates. The writer has examined these, and could find only the well-

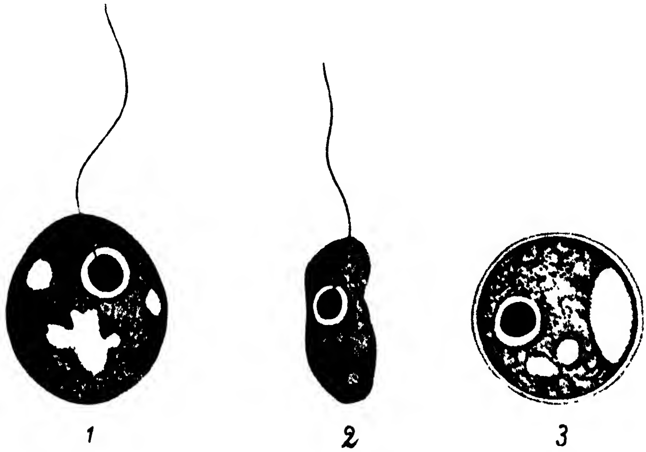


FIG. 139.—*Craigia migrans* (× *ca.* 3,000). (AFTER KOFOID AND SWEZY, 1921.)
1. Rounded type of flagellate. 2. Elongate type of flagellate. 3. Encysted form.

known forms. In several of the films *Chilomastix* was present. This flagellate, which is evidently quite common in Honduras, was not identified by Barlow, so it is not improbable that he mistook this organism for *C. hominis*.

The extensive investigations made during the war have cleared up many doubtful points in connection with the intestinal Protozoa of man, but neither *C. hominis* nor *C. migrans* has been rediscovered. The writer has long held the opinion that no such parasites of the human intestine exist, and in this conclusion he is in agreement with Dobell (1919). Kofoid and Swezy (1921*b*), as stated above, have claimed to have observed cases of infection with both species of *Craigia*. The parasite seen by these observers does not in its amœboid phase resemble *E. coli* in any way, while in the flagellated stage the flagellum is an exceedingly fine structure which is difficult to detect, and does not show the least resemblance to the tail-like processes figured by Craig. The organism corre-

sponds in every way with *Sphæromonas communis* described by Liebetanz from the rumen of cattle (Fig. 139). There seems, therefore, to be no doubt that Kofoid and Swezy have discovered a small uniflagellated organism in human fæces. The flagellum cannot be detected in every one of the organisms, but when it is present it arises from a granule near the surface of the parasite, while a fibre is depicted as connecting this granule with the centrally placed karyosome of the nucleus. In its course it passes through the nuclear membrane. Spherical cysts which contain a uninucleated cytoplasmic body and resemble the cysts of some free-living amœbæ also occur. The discovery of such a form does not in any way establish the authenticity of the genus *Craigia*. Kofoid and Swezy state that they have seen their organism in six cases. It is possible that, owing to the extreme fineness of the flagellum and the difficulty of detecting it except in well-stained specimens, this organism has sometimes been mistaken for *Endolimax nana*, to which it bears a superficial resemblance. On the other hand, the possibility of its being a coprozoic flagellate of the genus *Oikomonas* which has developed in the fæces after they have left the body has to be considered.

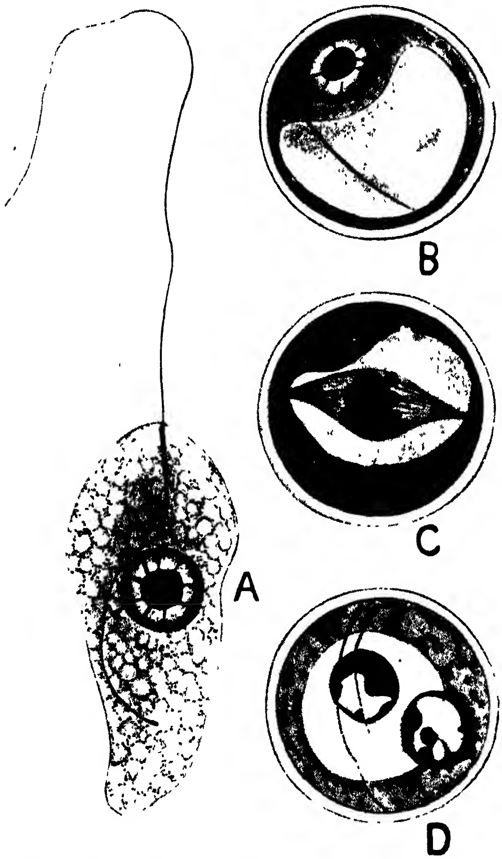


FIG. 140.—*Rhizomastix gracilis* ALEXEIEFF, 1911, FROM INTESTINE OF LARVA OF *Tipula* sp. (× 4,000). (AFTER MACKINNON, 1913.)

A. Flagellate form.
B. Cyst with one nucleus.
C. Cyst showing nuclear division.
D. Cyst with two nuclei.

Genus: Rhizomastix Alexeieff, 1911.

The flagellates of this genus have rounded or pear-shaped bodies and a central nucleus. There is a long flagellum arising from the anterior end of the body, and its axoneme is continued into the cytoplasm in the form of a long fibre which terminates in a blepharoplast behind the nucleus.

Rhizomastix gracilis Alexeieff, 1911.—This flagellate (Fig. 140), which varies in length from 6 to 11 microns, has been described from the intestine of axolotls by Alexeieff (1911), and by Mackinnon (1913) from tipulid larvæ. It has the structure described above, and produces spherical cysts, within which nuclear division occurs.

Yakimoff and Kolpakoff (1921) described as *Pararhizomastix hominis* a flagellate isolated by them from human fæces planted on agar media. The organism closely resembles Alexeieff's *Rhizomastix agilis* of the axolotl. The authors do not state the grounds on which they create the new genus, nor why they regard the flagellate as a human parasite, and not a coprozoic organism, which it undoubtedly is.

Genus: **Proleptomonas** Woodcock, 1916.

This genus was founded by Woodcock (1916) for a flagellate which he discovered in cultures from fæces of goats (Fig. 141). On account of its resemblance to the leptomonas of insects, it was placed by him in a new genus, *Proleptomonas*, of which there is one species.

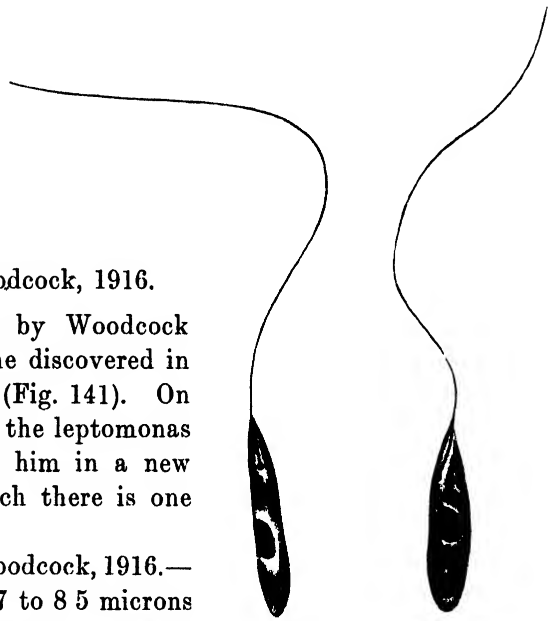


FIG. 141. — *Proleptomonas fæcicola:* A COPROZOIC FLAGELLATE FROM FÆCES OF GOATS (× 3,000). (AFTER WOODCOCK, 1916.)

Proleptomonas fæcicola Woodcock, 1916.—This flagellate measures from 7 to 8 5 microns in length by 1·25 to 1·75 microns in breadth (Fig. 141). There is a long anterior flagellum 16 to 21 microns in length and a central nucleus, in front of which is a blepharoplast from which arises the axoneme of the flagellum. Woodcock thinks it possible that *P. fæcicola* may be the present-day representative of the ancestral type from which the parasitic flagellates of the genus *Leptomonas* were derived. Fantham (1922) has seen a similar flagellate in decomposing cabbage, and, owing to the fact that a definite kinetoplast was present, he regards it as differing from Woodcock's *Proleptomonas.* He gives it the name *Herpetomonas brassicæ.* Another form found in soil he names *H. terricolæ.* These flagellates, however, do not belong to the genus *Herpetomonas,* and it is not improbable that they are identical with *Proleptomonas fæcicola.*

B. MONADIDÆ WITH TWO FLAGELLA.

Many free-living flagellates provided with two flagella have been described. Such are the various flagellates placed by Stein in the genus *Monas.* These are minute organisms which occur in stagnant water. They have ovoid or elongate amœboid bodies and two flagella, the thinner one of which is about twice the length of the other. There is no cytostome, but a contractile vacuole is present. They produce minute spherical cysts.

Yakimoff and Solowzoff (1921*a*) identified as *Monas vulgaris* a flagellate they obtained by inoculation of agar plates with human fæces in Russia. Yakimoff and his co-workers seem to believe that this affords sufficient evidence of parasitism in the human intestine. The organism is undoubtedly a free-living form which in the encysted condition contaminated the stool after it had been passed.

Genus: Heteromita Dujardin, 1841.

This genus was established by Dujardin for certain flagellates which had hitherto been included in the genera *Monas* or *Bodo,* and which possessed pear-shaped bodies provided with two anterior flagella, one of which was two or three times as long as the other. The longer flagellum, which was finer than the shorter one, could function as a trailing flagellum. It seems not improbable that the flagellate for which Krassilstschik (1886) created the genus *Cercobodo* and that for which Klebs (1892) proposed the name *Dimorpha* really belong to the genus *Heteromita.* Several flagellates of this genus were studied by Dallinger and Drysdale, for one of which Kent (1880–1882) proposed the name *Heteromita uncinata.*

The genus *Heteromita* can be defined as including minute flagellates which have pear-shaped bodies from the more pointed anterior end of which arise two flagella of unequal length. The shorter one, which may be thicker than the other, is from once to twice the length of the body and is directed forwards. The finer and longer flagellum may be two to four times the length of the body. It performs lashing movements, and when in contact with a surface may act as a trailing flagellum. The axonemes of the flagella, which commence in blepharoplasts on the nuclear membrane, pass to the anterior end of the body, and thence directly into the flagella. There is no cytostome, and a contractile vacuole is present in the posterior region of the body. The body is exceedingly amœboid when in contact with a surface. In this condition the flagella may be lost, the flagellate then moving about like a small amœba.

Reproduction is by longitudinal fission of free-swimming forms, or

rounded individuals with or without flagella. Encystment in spherical cysts occurs. The genus *Cercomonas* (see p. 629) contains flagellates of

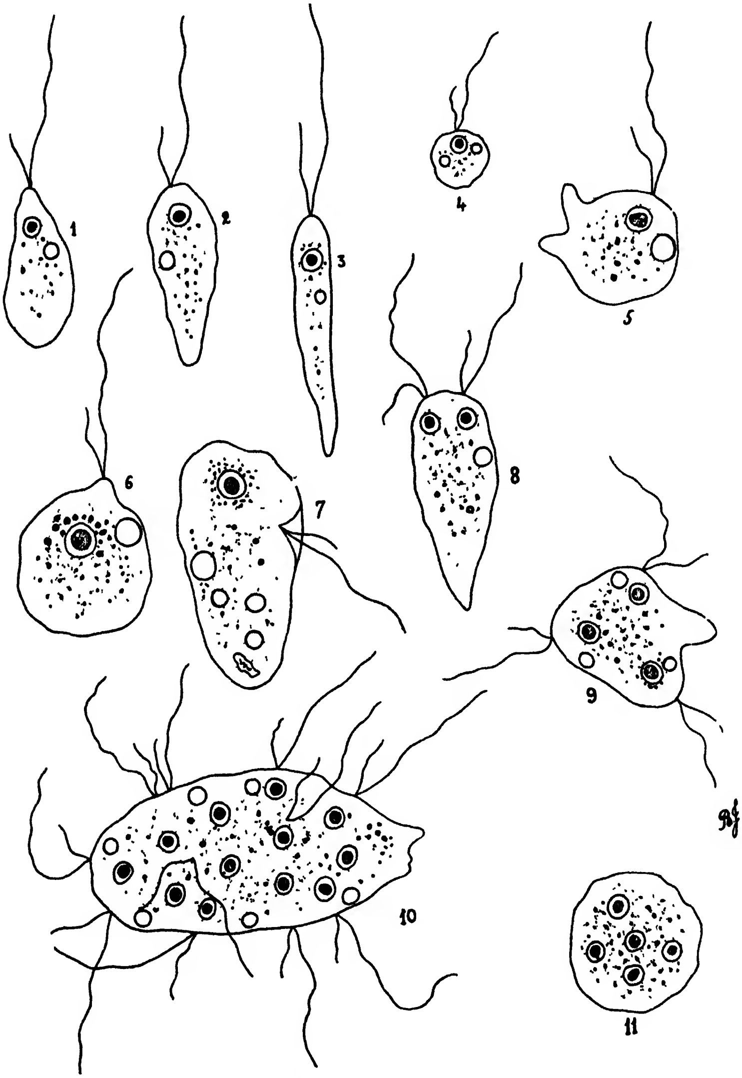


FIG. 142.—*Heteromita uncinata* FROM CULTURE ON AGAR (× 4,000). (ORIGINAL FROM LIFE.)

1-7. Various types of flagellate with single nucleus, and contractile vacuole.
8. Binucleated dividing form. 9. Trinucleated form with three contractile vacuoles.
10. Multinucleated form with many contractile vacuoles.
11. Form without flagella (five nuclei).

very similar structure, except that the axoneme of the trailing flagellum passes over the surface of the body from the anterior to the posterior end

before entering the flagellum. The genus *Bodo* includes flagellates which also have two flagella, but the axonemes of these arise from two blepharoplasts which are separated from the nuclear membrane, and which have a parabasal body associated with them. There seems to be no justification whatever for Nöller's (1922) inclusion in the same genus of flagellates of the *Cercomonas* type with adherent axoneme and those of the *Heteromita* (*Cercobodo*) type, much less for his assumption that they all belong to the Rhizopoda.

Heteromita uncinata Kent, 1880.—This was one of the flagellates studied by Dallinger and Drysdale (1873), who accurately described the main features of its life-cycle. What appears to be this organism was seen by the writer as a coprozoic flagellate in old human fæces. It is pear-shaped, with a rounded posterior end and somewhat pointed anterior end (Fig. 142). It varies in length as a rule from about 3 to 8 microns, but exceptionally large forms up to 10 microns in length occur. A contractile vacuole is present in the hinder region of the body, and there is no cytostome. Arising from the pointed anterior end are two flagella of unequal length. The shorter, which is slightly thicker than the other, is approximately as long as the body, and directed forwards during progression. The longer flagellum, two to four times the length of the shorter one, performs wide sweeping movements in front of the flagellate when it is swimming freely. If the long flagellum comes in contact with the glass, the flagellate still moves forwards by the action of the shorter flagellum, while the long one trails behind over the surface. In stained individuals, the axonemes of the two flagella can be traced to the surface of the nuclear membrane, which may be drawn out into a cone at the point of union (Fig. 68). In some individuals, two blepharoplasts can be distinguished at the apex of the cone. The centre of the nucleus is occupied by a large karyosome. In the free-swimming condition the body of the flagellate retains its pear shape, but if it comes in contact with a surface it exhibits amœboid changes of shape. In pure cultures reared from a single flagellate there occur amœboid forms devoid of flagella, so that a definite amœboid phase has to be recognized. When grown on agar plates there occur much larger multinucleated forms, with a corresponding number of contractile vacuoles and pairs of flagella. Dallinger and Drysdale described the fusion of numerous flagellates to form a multinucleated body. That the multinucleated forms which occur on agar plates do not always arise in this way is shown by the fact that, after staining, they may have all their nuclei in process of division, the body containing a number of spindles. These multinucleated forms, as they occur on agar plates, are to be regarded as instances of delayed division of the cytoplasm.

The flagellate reproduces by longitudinal division in the free-living

condition, but most usually after having become spherical and quiescent. The amœboid forms without flagella also multiply by binary fission. The blepharoplasts on the nuclear membrane divide, and the two pairs of daughter blepharoplasts take up positions at opposite poles of the elongating nuclear membrane (Fig. 68). Two new axonemes grow out from two of the daughter blepharoplasts, and these form flagella at the surface of the body as division is proceeding. The karyosome of the nucleus breaks up, and a small number of chromosomes appears at the equator of the spindle which forms within the nuclear membrane between the blepharoplasts. Daughter plates are formed by division of the chromosomes, and a long spindle stretches across the elongated body of the flagellate. The daughter plates approach the blepharoplasts, the intermediate part of the spindle disappears, and the nuclear membrane closes round the daughter chromosomes, which concentrate into the characteristic karyosomes. The cytoplasm now becomes constricted and divided into two parts, and two flagellates result. Division of the amœboid forms takes place in the same manner except for the absence of flagella.

Under adverse conditions the flagellate loses its flagella, becomes spherical and encysts in spherical cysts 3 to 6 microns in diameter. The cyst wall appears perfectly smooth and shows no indication of pores. On agar plates larger spherical, ovoid, or more irregularly shaped cysts up to 10 microns in diameter occur. As the included cytoplasm may contain as many as sixteen nuclei, it is probable they are formed by the encystment of the multinucleated forms which occur in these cultures. The emergence from the cysts of large numbers of minute flagellates and the conjugation of two individuals, as described by Dallinger and Drysdale, were not observed.

The life-history and structure of this flagellate is of interest in that it closely resembles *Cercomonas longicauda*, another coprozoic organism (Fig. 259). It differs chiefly in the fact that both flagella arise at the anterior end of the body, there being no tendency for one of the axonemes to pass along the surface of the body before entering a flagellum. The amœboid forms of *C. longicauda* retain the two flagella, while those of *H. uncinata* usually discard them.

It seems not improbable that the flagellate which Sangiorgi (1922*a*) cultivated from human fæces, and which he named *Pirobodo intestinalis*, belongs to the genus *Heteromita*. As described, it had a pear-shaped body with two long flagella arising from the pointed anterior end. The dimensions given are 12·8 to 16·6 microns for the length and 9·6 to 14·4 for the breadth. The description is, however, so inadequate that it is impossible to identify the organism with any degree of accuracy.

Genus: Dimastigamœba Blochmann, 1894.

There is some question as to whether the organisms included in this genus should be regarded as Mastigophora or Rhizopoda (Figs. 61, 120, 121). It appears that the great part of their existence is spent as amœbæ, in damp soil or similar situations, but that at certain times, when excess of fluid is suddenly added to the medium, they temporarily assume a flagellated condition. Two flagella are developed, the body becomes elongated, and the organism has the characters of a typical member of the Mastigophora. After leading a free-swimming existence for about a day the flagella are lost, and the amœboid condition is again assumed. The axonemes of the two flagella appear to be connected with the nuclear membrane, and in this respect the flagellates resemble those of the genus *Heteromita.* Division, however, takes place only in the amœboid phase, during which the nucleus divides in a characteristic manner, differing in this respect from the method of nuclear division of *Heteromita.* Furthermore, the spherical cysts are provided with a number of pores which render them easily recognizable. This genus has been considered more fully in the group of flagellated amœbæ (p. 262).

Genus: Spiromonas Perty, 1852.

This genus includes flagellates which in the adult condition have narrow elongate bodies which are spirally twisted. There are two flagella, which arise from the anterior end of the body near a small cytostome. Two blepharoplasts lie near the insertion of the flagella and the nucleus is centrally placed. When reproduction takes place, the body becomes spherical and enclosed in a cyst, within which division into daughter flagellates takes place.

Spiromonas angusta (Dujardin, 1841).—This organism, which in the adult stage has an elongate spiral body 12 to 13 microns long by 1·75 to 2 microns broad, was referred to by Dujardin (1841) as *Heteromita angusta* and Stein (1878) as *Bodo gracilis.* Kent placed it in the genus *Spiromonas* (Fig. 143). It was studied by Woodcock (1916) in cultures made from goat's fæces. The smallest forms have ovoid bodies measuring 2·5 by 1 micron, and provided with two backwardly directed flagella, which may be more than three times the length of the body. As growth takes place the body becomes definitely spiral, while with further development it becomes bean-shaped. In the largest forms the flagella are about as long as the body, which undergoes no change in shape and appears to be quite rigid. The nucleus is in the anterior half of the body, while the axonemes of the flagella arise from two blepharoplasts in front of the nucleus. Multiplication occurs only in the encysted condition, which is

brought about by the flagellate losing its flagella and becoming spherical. Within the cyst it divides usually into three, but sometimes into two or four small forms, which develop flagella and escape from the cyst. Syn-

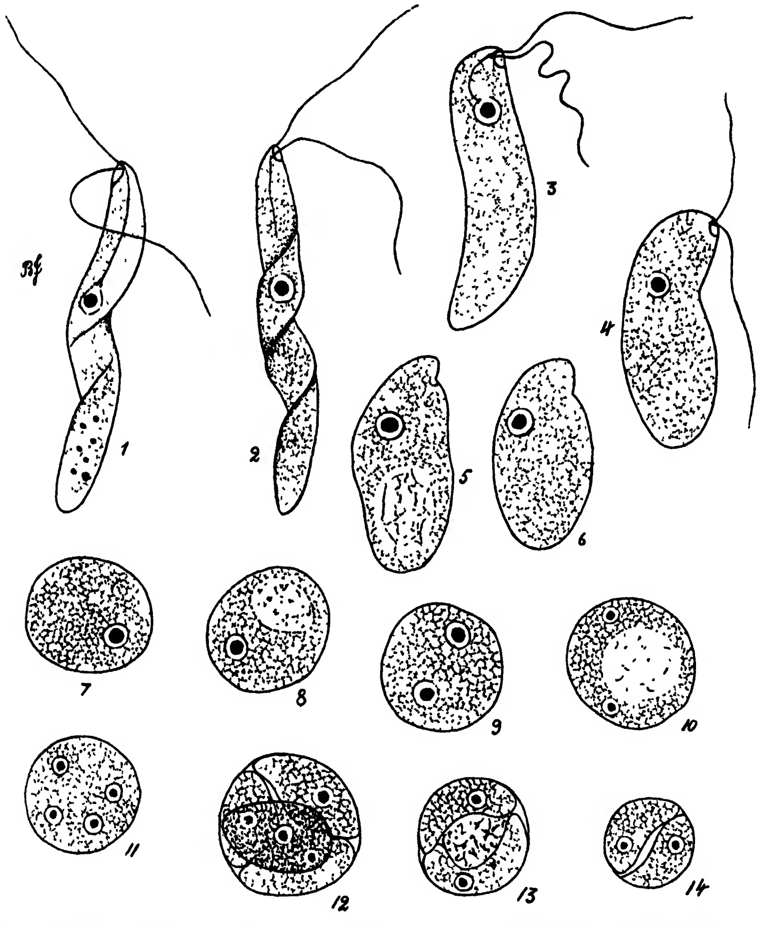


FIG. 143.—*Spiromonas angusta* : A COPROZOIC FLAGELLATE FROM PIG'S FÆCES (× 3,000). (ORIGINAL.)

1-2. Narrow forms showing spiral groove.
3-4. Thicker forms in which spiral groove is not evident.
5-6. Forms without flagella retracting for encystment; one has a large vacuole.
7-8. Encysted forms with one nucleus; one has a vacuole.
9-10. Similar forms with two nuclei.
11. Encysted form with four nuclei.
12. Encysted form after division into four; each develops two flagella and escapes from the cyst.
13-14. Encysted forms after division in two.

gamy was also observed to take place. Two individuals may form a common cyst, within which they unite, or they may first unite and form a cyst afterwards. A coprozoic *Spiromonas* has been seen by the writer in

pig's fæces. Though certain individuals reached a length of 15 microns, the flagellate is probably identical with that studied by Woodcock. In the younger forms the body is distinctly flattened, and resembles a blade of grass twisted into a spiral. There is a small but definite cytostome, though Woodcock stated that no cytostome was present in the form studied by him. The two flagella arise from the region of the cytostome, one apparently from its anterior lip and the other from a point within it. In some individuals a thread, which may be the axoneme, can be traced to the membrane of the spherical nucleus which occupies a central position in the body. In some individuals there appears to be a granule at the base of each flagellum near or on the surface of the body. The older individuals become more cylindrical in form, though they may still show

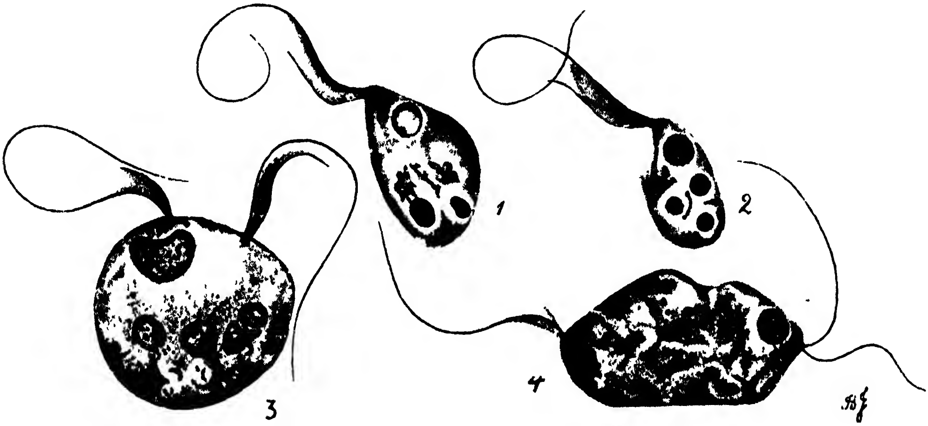


Fig. 144.—*Phyllomitus undulans* : A Coprozoic Flagellate in Goat's Fæces (× 3,000). (After Woodcock, 1916.)

1-2. Ordinary type of flagellate. 3-4. Dividing forms.

indications of a spiral twist. In preparation for division the body gradually retracts to a spherical form and encysts. The nucleus divides to form two nuclei, and these again to give rise to four. The body then divides into four daughter flagellates. In some cases two and in others three daughter flagellates are formed.

Sangiorgi (1917) described as *Toxobodo intestinalis* a small flagellate he had cultivated from human fæces. Its measurements were 8 to 9·6 microns by 3·2 to 4·8 microns. From the figures, it appears that the flagellate was probably a *Spiromonas*. Both it and the one named *T. sangiorgii*, and cultivated from mouse fæces by Yakimoff (1925), are probably *S. angusta*. Similarly, the coprozoic flagellate seen by Alexeieff (1918) in the fæces of the horse and tortoise, and which he named *Alphamonas coprocola*, is probably the same spiral organism, as pointed out by Woodcock (1921),

Genus: Phyllomitus Stein, 1878.

This genus includes *Phyllomitus undulans*, which was originally described by Stein. Woodcock (1916) obtained it in culture from goat's fæces. It has an ovoid body, and varies in size from 6 to 13 microns by 3 to 8 microns (Fig. 144). There are two flagella, one about twice the length of the body and the other less than half this. The two flagella are united by a membrane. Multiplication is by binary fission.

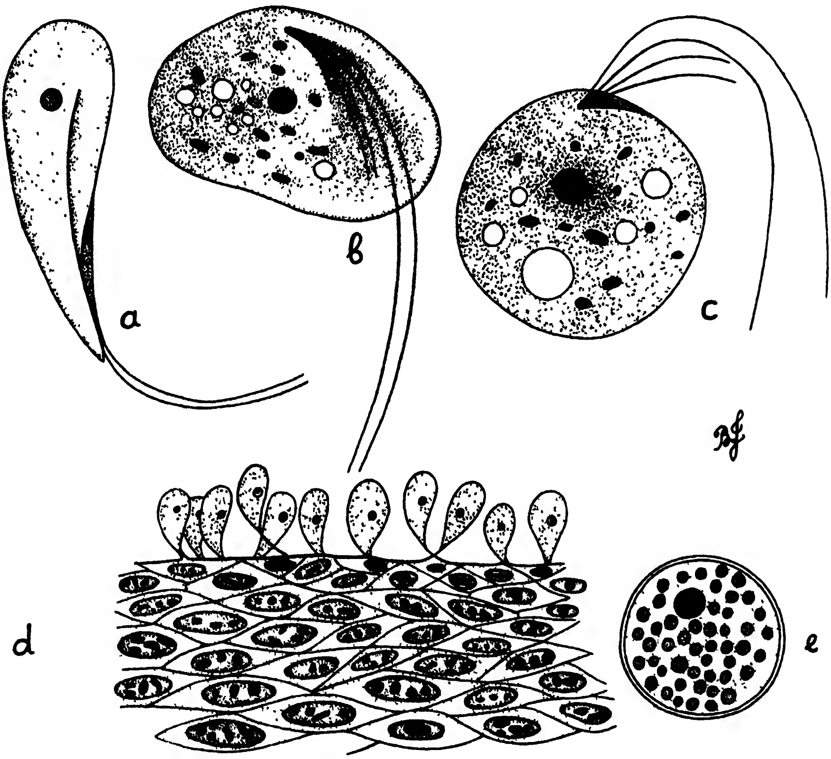


FIG. 145.—*Costia necatrix* FROM SKIN OF FISH. (AFTER MOROFF, 1903.)

a. Side view of flagellate (× 2,000).
b-c. Probable division forms with two new flagella developing (× 2,000).
d. Section of skin with attached flagellates. *e.* Encysted form (× 2,000).

Genus: Costia Leclerq, 1890.

This genus was founded by Leclerq (1890) for a flagellate which is parasitic on the skin of fish. The organism is pear-shaped, and has two or four flagella arising in a groove.

Costia necatrix (Henneguy, 1883).—This flagellate, the only member of the genus, was discovered by Henneguy (1883, 1884). He placed it in the genus *Bodo* as *Bodo necator*, while Leclerq (1890) created for it the new genus

Costia. The body, which is somewhat flattened, is pear-shaped in outline (Fig. 145). At the anterior pointed end is a funnel-like depression, from the bottom of which arise the flagella. According to Moroff (1903), there are two long flagella which extend beyond the body and two short ones confined to the interior of the funnel. It seems possible that the two short flagella are new ones forming preparatory to division, and that the organism has really only two long flagella. The body measures from 10 to 20 microns in length by 5 to 10 microns in breadth. The parts of the flagella beyond the body have a length slightly shorter than that of the body itself. There is a spherical nucleus at the middle of the body, while behind it is a contractile vacuole. Reproduction is by longitudinal division, while spherical cysts 7 to 10 microns in diameter are formed. The flagellates are parasitic on the skin of fish, to which they are attached by their flagella. They sometimes occur in enormous numbers on young fish artificially reared, and have been suspected of causing a high rate of mortality.

C. MONADIDÆ WITH THREE FLAGELLA.

Genus: Enteromonas Fonseca, 1915.

This genus was founded by Fonseca for a flagellate named by him *Enteromonas hominis* which he found in human fæces in Brazil (Fig. 146). The various descriptions he has given of the organism are not in agreement. The last account given by him (1920) describes the flagellate as having a spherical body 5 to 6 microns in diameter. There was a nucleus near the anterior end 1 micron in diameter. Running from the nucleus to the anterior end of the body was an axoneme which terminated in a blepharo plast, from which arose three flagella. There was no cytostome. The cytoplasm contained food vacuoles, but no other structures. Encysted forms were not encountered. Chalmers and Pekkola (1917*a*, 1918) recorded the finding of an organism in human fæces which they believed to be identical with Fonseca's *E. hominis,* while Chatterjee (1917) erroneously ascribed to the genus *Monocercomonas* an organism with similar structure from human beings in India. Later in the same year (1917*a*) he gave an account of a new organism which he named *Trichomastix hominis,* on account of the fact that some of the individuals had four free flagella. It is very probable that the forms with four flagella were producing new flagella in process of division, for, of the thirty-five individuals figured, only four are shown with four flagella. All the others have three, with the exception of one with two. It seems clear, therefore, that the organism is not a *Trichomastix* (*Eutrichomastix*) at all, and that it is the same as the form previously described by him as *Monocercomonas,* which again appears to be identical with the forms first seen by Fonseca. The

flagellate described by him (1919) as *Enteromonas Bengalensis* is possibly the same organism, though some of the figures suggest *Embadomonas intestinalis.* Leger, M. (1918*a*), described a similar form from man in Guiana, and regarded it as *E. hominis.* These various accounts agree in describing *E. hominis* as a flagellate with rounded body, three flagella, and no cytostome. Yakimoff (1925) gave the name *Enteromonas fonsecai* to a form in the guinea-pig.

There seems to be considerable doubt as to the accuracy of the descriptions of the genus *Enteromonas.* Dobell and O'Connor (1921) suggest that the various observers were actually dealing with *Tricercomonas intestinalis*, and that the fourth posterior flagellum had been overlooked (Fig. 261). It appears, however, that another and more probable explanation can be found. As described by the writer (1910*b*), *Chilomastix mesnili* may occur as a small spherical flagellate with three anterior flagella (Fig. 256, 7, 8). In these forms the cytostomal groove and its enclosed flagellum may be difficult to detect or quite invisible, so that flagellates appear to have the structure ascribed to *Enteromonas hominis.* This mistake appears to have been made by Chalmers and Pekkola, for the writer has been able to examine their original films. There can be no doubt that their *E. hominis* is merely a small rounded form of *C. mesnili.* Though they state that the latter flagellate was never found in association with *E. hominis*, the writer has found typical forms in the films. In their description of *C. mesnili*, these observers (1918) draw attention to the small round forms with obscured cytostomal groove, but it did not occur to them that their *E. hominis*, previously described, might be the same forms. It is not improbable that Fonseca and other observers were also describing as *Enteromonas* the small forms of *C. mesnili.* If this be so, then the name *Enteromonas* becomes a synonym of *Chilomastix.* Fonseca (1918) described as *E. intestinalis* of the rabbit a flagellate which was said to have the same structure as his *E. hominis* of man. This form, as well as the human one, was seen by da Cunha and Pacheco (1923), who also saw another in the viscacha in Brazil. If the human form is a *Chilomastix*, it is not improbable that the rabbit one is also, as it is liable to infection with a species of *Chilomastix.* Lynch (1922*a*) has, however, obtained from the guinea-pig a culture of a flagellate having the structure ascribed to *Enteromonas.* It apparently showed no tendency to develop into *Chilomastix*, with which the guinea-pig may also be infected,

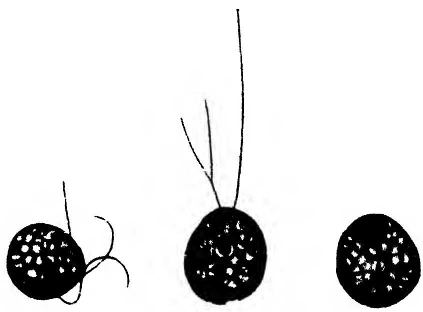


FIG. 146.—*Enteromonas hominis* (× *ca.* 2,000). (AFTER FONSECA, 1916.)

but remained as a small rounded organism with three flagella, one of which sometimes functioned as a trailing flagellum. Whether this flagellate should be regarded as belonging to the genus *Enteromonas* depends on whether Fonseca's human *Enteromonas* was or was not a *Chilomastix.* Brug (1923) gave a description and figures of *E. hominis* in Sumatra. Some of the figures are suggestive of *Tricercomonas intestinalis*, and as Jepps (1923) had encountered this flagellate in Malaya, it seemed possible that Brug was actually dealing with it. Brug has kindly allowed the writer to see preparations of his flagellate, which is undoubtedly *T. intestinalis.*

The determination of the structure of small flagellates in fæces is an exceedingly difficult procedure. A careful observation of living individuals, or those which have been killed by exposure to osmic vapour or iodine solution, will often yield more information than the study of fixed and stained films.

D. MONADIDÆ WITH FOUR FLAGELLA.

A number of coprozoic or intestinal flagellates have been described which possess four anterior flagella and no accessory structures in the cytoplasm beyond the nucleus and blepharoplasts. These have been placed in various genera, but it is very doubtful if many of these are valid. Their classification is rendered difficult by members of the genus *Eutrichomastix* (p. 671), which correspond in structure except for the possession of an axostyle. Other flagellates having a similar structure, but possessing a fibre in the place of a true axostyle, belong to the genus *Retortamonas* (often called *Monocercomonas*, p. 677). The axostyle or the fibre may not be visible, in which case the flagellates resemble Monadidæ with four flagella. It is evident that when an organism is seen with four flagella and no axostyle or fibre, it may be a *Eutrichomastix* or *Retortamonas* in which these structures are not visible, or a true Monad with four flagella.

Genus: Tetramitus Perty, 1852.

This genus was created by Perty (1852) for certain free-living, pear-shaped flagellates, which possess a cytostome and four flagella, one of which might be a trailing flagellum. There are several species recorded by Perty, Klebs, and others. It is possible that some of the flagellates with four flagella which occur in fæces belong to this genus. The form described by Dobell (1908*c*) as *Monocercomonas bufonis* from the toad is a pear-shaped organism 12 to 15 microns in length and 3 to 6 microns in breadth. There is no cytostome. The nucleus lies near the blunt end of the body, and in front of it are four blepharoplasts from which arise four flagella, all of which are directed forwards. There was no axostyle or fibre in the body. This flagellate evidently does not belong to the genus *Monocercomonas* (*Retortamonas*), as the axial fibre is absent. It may belong

to the genus *Tetramitus*, as Dobell suspected, though the absence of cytostome is against this view.

As noted above, the *Monocercomonas* described by Chatterjee (1917) from the human intestine probably has only three flagella, and not four, and should not be included in this genus.

Aragão (1916) established the new genus *Copromastix* for a flagellate with four anterior flagella, which appeared in cultures of human and rat fæces in egg-albumen water (Fig. 148). The organism is pear-shaped, with a blunt anterior end, at one side of which is a cytostome. The length of the body is 16 to 18 microns and the breadth 7 to 9 microns. Smaller forms, however, occur. The flagellates multiply by binary fission. The blepharoplast from which the four flagella take origin first divides into two, some of the four flagella remaining with one portion and the others with the other portion. The nucleus divides by mitosis, and this is followed by division of the body. New flagella are formed from the blepharoplast till each daughter individual has four. Aragão names the flagellate *Copromastix prowazeki*, but it corresponds very closely with *Tetramitus rostratus*, a free-living form first seen by Perty (1852). It is probable that it as well as *Copromastix aragaoi* cultivated from human fæces by Yakimoff (1925) are actually this species.

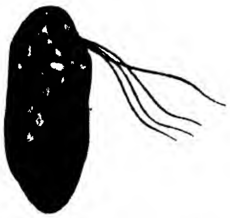


FIG. 147.—*Chilomitus caviæ* FROM THE CÆCUM OF THE GUINEA-PIG (× *ca.* 2,000). (AFTER FONSECA, 1916.)

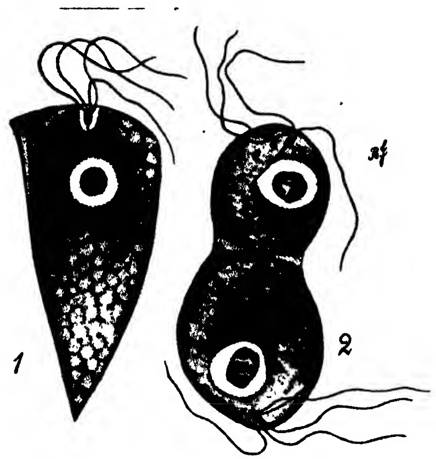


FIG. 148.—*Copromastix prowazeki:* A COPROZOIC FLAGELLATE APPEARING IN CULTURES OF HUMAN AND RAT FÆCES (× *ca.* 3,000). (AFTER ARAGÃO, 1916.)

1. Usual type. 2. Dividing form.

Bunting (1922) obtained by culture from the cæcal contents of rats an amœba which after reproduction in this form became transformed into a flagellate of the *Tetramitus* type, with four flagella, a lateral cytostome, and contractile vacuole. After reproduction in the flagellate stage had taken place, reversion to the amœboid phase occurred. Spherical cysts 6 to 18 microns in diameter were produced by the amœbæ. Rats and mice

were fed on the cultures. When examined *post mortem* one to five days later, no amœbæ or flagellates could be found in the intestine, but cysts were present. Culture from the intestinal contents again gave cultures of amœbæ and flagellates. It is evident that the flagellate and the amœba are coprozoic, and that they represent different phases of development of one organism. The flagellate phase as figured by Bunting has a striking resemblance to *Copromastix prowazeki*. It is possible that these organisms are related to *Dimastigamœba*, which also has both a flagellate and an amœboid phase (see p. 262).

Fonseca (1916) created the genus *Chilomitus* for a flagellate of the cæcum of the guinea-pig (*Cavia porcellus* and *C. aperea*) in Brazil. It is said to vary in size between two extremes. The large form is 12 to 17 microns in length by 4 microns in breadth, and the small one 8 to 10 microns in length by 4 to 5 microns in breadth (Fig. 147). The anterior end is rounded and the posterior end tapering, while the body itself is very rigid owing to a well-developed ectoplasmic layer. There are four anterior flagella which arise from a blepharoplast and a cytostome which is much shorter than that of *Chilomastix*. There is no flagellum within the cytostome. The nucleus near the flagellar origin is not vesicular, and appears to consist of a mass of granules. The margins of the cytostome are not stiffened by marginal filaments as in *Chilomastix*.

Lavier informs the writer that he has seen this flagellate, which was named *Chilomitus caviæ* by Fonseca, in the rodent *Viscacia viscacia* of the Argentine. In addition to the structures noted by Fonseca he has seen an axial fibre passing longitudinally through the body.

Chalmers and Pekkola (1918) created the genus *Protetramitus* for a flagellate which was described as having a spherical body and four flagella arising from blepharoplasts, near which was the single nucleus. The flagellate *Protetramitus testudinis* was found in the tortoise (*Testudo calcarata*). The writer has examined the films, and finds that, in addition to *Entamœba testudinis* and *Balantidium testudinis*, a number of flagellates are present—*Hexamita*, *Trichomonas*, *Eutrichomastix*—and a large organism with a single flagellum which appears to belong to the genus *Oikomonas*. Unfortunately, owing to faulty technique, the majority of the organisms are imperfectly fixed, and many have actually dried in the film. The result is that many of the *Trichomonas* and *Eutrichimastix* have become rounded, while the axostyles, supporting filament and undulating membrane, are not visible. It is easy, however, to trace every degree of this change between the flagellates which were named *Protetramitus* by Chalmers and Pekkola and the typical *Trichomonas* and *Eutrichomastix*. The flagellate named *Protetramitus testudinis* is thus nothing more than a rounded and altered *Trichomonas* or *Eutrichomastix*.

E. MONADIDÆ WITH MORE THAN FOUR FLAGELLA.

Of flagellates with more than four flagella, the only genus which should be mentioned is *Callimastix* Weissenberg, 1912. The genus was created by Weissenberg for a flagellate parasitic in the body cavity fluid of a species of *Cyclops*. He gave it the name *Callimastix cyclopis*.

A very similar form discovered by Braune (1913) in the rumen of cattle was named by him *C. frontalis* (Fig. 149, A). The body, which is spherical or ovoid, has a diameter of about 12 microns, and possesses a single nucleus with large central karyosome. The characteristic feature of the flagellate is its possession of a number of flagella which spring from a row of blepharoplasts. The flagella are arranged in one plane, and appear as if united laterally to form a band about 30 microns in length. This organism was seen by Fonseca (1916) in cattle, sheep, and goats in South America. He established a new family, Callimastigidæ.

An organism which may be related to *Callimastix frontalis* is *Selenomonas palpitans*, which was described by Simons (1921) from the cæcum of guinea-pigs (Fig. 149, B). It seems probable that *Ancyromonas ruminantium*, which Certes (1889) found in the rumen of cattle, is the same organism. Prowazek (1913*a*), as Kerandel (1909) had done before, saw it in the blood-films from African antelopes, and concluded that these had been contaminated from the intestine. He created the genus *Selenomonas*. Soon after Woodcock and Lapage (1913) observed it in the stomach of goats, and placed it in a new genus as

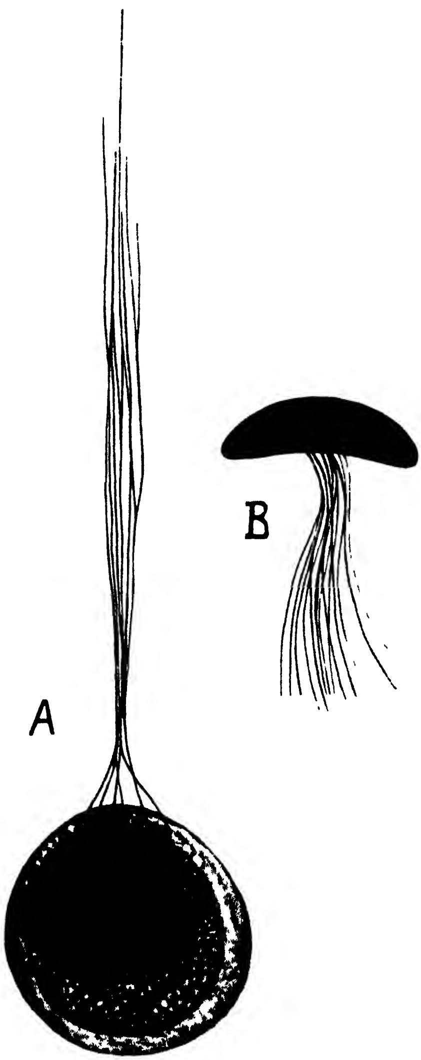


FIG. 149.—(AFTER FONSECA, 1916.)

A. *Callimastix frontalis* from rumen of ox, sheep, and goat (× *ca.* 4 ,000).
B. *Selenomonas ruminantium* from cæcum of wild guinea-pig (× *ca.* 4,000).

Selenomastix ruminantium, realizing that it was the same as the organism described by Certes. Prowazek's name evidently has priority, as the organism certainly does not belong to Kent's genus *Ancyromonas*. Da Cunha (1915) noted the organism in the cæcum of guinea-pigs, as did also Fonseca (1916) and Simons (1920, 1921). The form in the guinea-pig was described in detail by Boskamp (1922). The body of the organism is a rigid crescent measuring 6·8 to 9·1 by 1·8 to 2·3 microns. A bunch of flagella springs from the hollow of the crescent, near which is a deeply staining mass. Reproduction is by transverse fission through the flagellar region. Half the flagella pass to each daughter individual. Boskamp believes that the organism is not a Protozoon, but is related to the *Spirilla*. The writer has seen the organism in large numbers in the cæcum of a guinea-pig in England. It seems quite possible that Fonseca's *Callimastix* is a rounded form of the same or a similar organism.

2. *Family:* TRYPANOSOMIDÆ Doflein, 1901.

In this family are grouped a number of closely related flagellates. They are the true trypanosomes typically seen in the blood of vertebrates or their invertebrate hosts; the leptomonas, crithidia, and herpetomonas, which have only an invertebrate host, in which they live mostly as intestinal parasites; the leishmania, which, like the trypanosomes, have both a vertebrate and an invertebrate host, though the latter is not definitely known; and the phytomonas, which have both an invertebrate and plant host.

RELATION OF VARIOUS TYPES TO ONE ANOTHER.

All the members of the family resemble one another in the possession of a nucleus and a single flagellum which arises from a composite structure, the kinetoplast (Fig. 150). The latter is made up of a posterior deeply staining body, the parabasal, and an anterior blepharoplast in which the axoneme of the flagellum has its origin. The kinetoplast, or the parabasal alone, is often termed the kinetonucleus, a name proposed by Woodcock (1906), while Laveran and Mesnil, in their writings, refer to it as the small nucleus or centrosome. It is also called the micronucleus, a name which should not be employed, for it is used to designate one of the highly specialized nuclei of the Ciliata. The term kinetonucleus implies that it is equivalent to a nucleus, and in this sense is misleading. On this account the term kinetoplast, first employed by Alexeieff (1917*b*) for the corresponding structure in *Bodo caudatus*, will be used here. The portion of the axoneme or axial filament of the flagellum between the blepharoplast and the surface of the body where the flagellum commences is often called the rhizoplast, but this term has been used for many different fibrils. The body of one of these flagellates usually consists of an elongated, flattened,

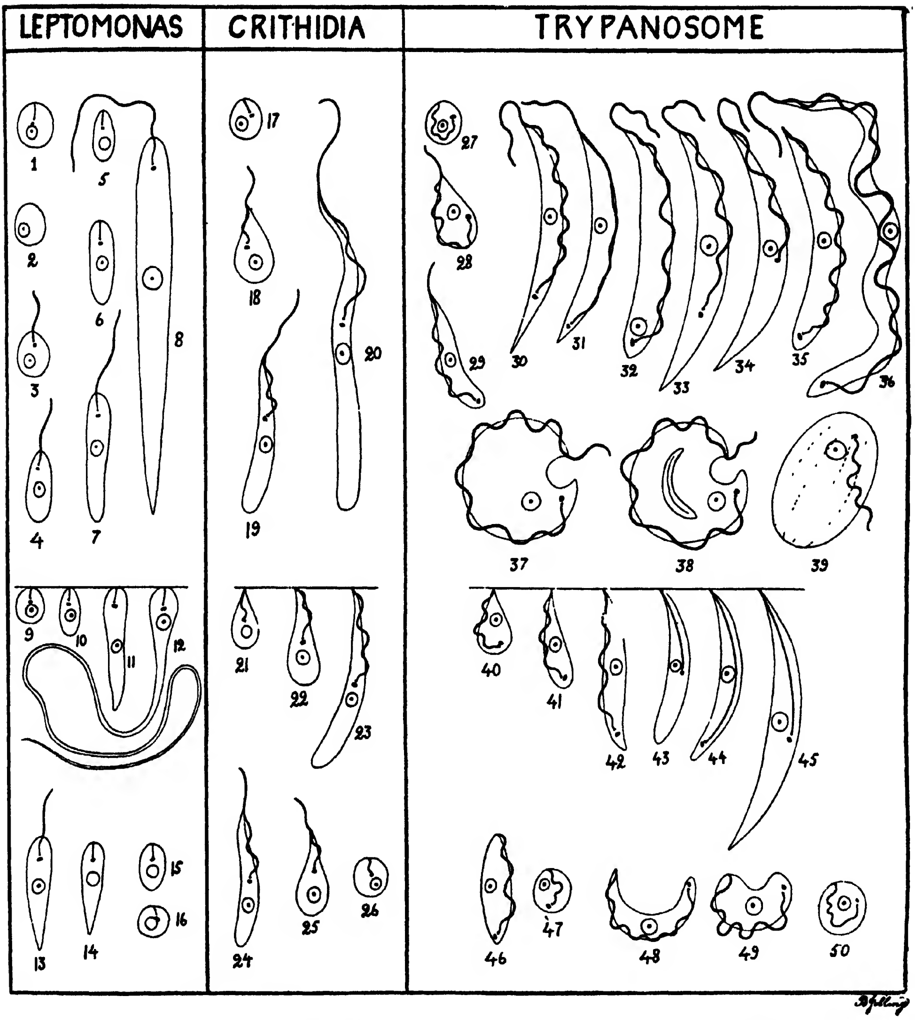


FIG. 150.—VARIOUS FORMS ASSUMED BY FLAGELLATES OF THE GENERA *Leptomonas* (1-16), *Crithidia* (17-26), AND *Trypanosoma* (27-50). (ORIGINAL.)

The lines represent the surface of epithelium to which flagellates become attached.

1-4 and 5-7. Evolution of round form (cyst) into leptomonas form 8.
9-11. Attached forms in which the flagella are lost.
12. Overgrown cercoplasma form.
13-16. Retrogression of leptomonas form into round form (cyst).
17-20. Evolution of round form (cyst) into crithidia form.
21-23. Attached forms in which flagella are lost.
24-26. Retrogression of crithidia form into round form (cyst).
27-29. Evolution of round form (cyst) into trypanosome form.
30-39. Various types of trypanosome form.
40-42. Attached forms in which flagella are lost, but undulating membrane is still present.
43-45. Attached forms in which flagella are lost and no undulating membrane present (Rhynchoidomonas forms).
46-47 and 48-50. Retrogression of trypanosome form to round form (cyst).

and curved blade of cytoplasm which is more or less tapering at each end. The surface of the body is covered by a very fine but denser layer of cytoplasm, the periplast. That such a fairly strong and resistant membrane is present Minchin (1909*a*), from a study of the cytology of *Trypanosoma lewisi*, considers obvious from the manner in which the trypanosomes retain their body form under trying circumstances. The cytoplasm has a finely alveolar structure, and very frequently a distinct vacuole occurs near the kinetoplast.

The simplest flagellate type is the leptomonas, which has an elongated and sometimes slightly curved blade-like body, and the various structures described above (Fig. 150, 8). All the other flagellates of this family may be regarded as having arisen from the leptomonas form. The first modification is the displacement backwards of the kinetoplast, which takes up a position near, but still anterior to, the nucleus. There is a considerable lengthening of the axoneme, which now, instead of passing through the body of the flagellate, takes a lateral course to the convex margin, and then passes along the surface of the body or on the edge of a cytoplasmic ridge—the undulating membrane—to the anterior end of the body, and thence into the flagellum. The free margin of the membrane, when one is present, is longer than the attached margin; hence it is thrown into folds, and has an undulatory movement when in action. Flagellates of this type are distinguished as crithidia (Fig. 150, 20). A further change occurs with continued displacement of the kinetoplast, which passes the nucleus and ultimately occupies a position near the posterior end of the flagellate. The axoneme then passes along the surface of the body for almost its entire length or along the margin of an undulating membrane, so that the trypanosome form is reached (Fig. 150, 30). These three flagellate types—leptomonas, crithidia, and trypanosome—may all of them transform in a converse manner to produce finally shorter and more rounded individuals till the leishmania form arises (Fig. 150, 1, 17, 27). The latter has a small round or ovoid body containing the nucleus and kinetoplast. There is no flagellum, but the axoneme can often be detected as extending from the kinetoplast to the surface of the body. These leishmania forms, under suitable conditions, will transform again into any one of the flagellate types from which they were originally derived; or, if they arise in the intestine of an invertebrate, as in the case of those flagellates which are limited to an invertebrate host, they may encyst and escape in the fæces.

Since purely insect flagellates are transmitted from one host to another by small encysted leishmania forms, it follows that the leishmania forms which escape from the cysts after they are ingested are much smaller than the fully-grown flagellate stages. In the intestine of the host these small leishmania forms increase in size, develop flagella, and change their shape

till the fully-developed flagellate stage is reached. Multiplication may take place at any period of this development. All the intermediate forms between the cyst and the elongate flagellates have been termed by Patton (1909) *pre-flagellates*. Conversely, in the hind-gut the flagellate forms, by a reverse process, become leishmania forms again. These eventually encyst and escape with the dejecta of the insect. The forms between the adult flagellates and the cyst have been styled *post-flagellates*. It is doubtful, however, if the cycle, as, for instance, that of *Crithidia gerridis* (Fig. 166), is as definite or simple as this nomenclature implies. If nutrition is lacking in the intestine, it may happen that flagellate forms become shorter and attach themselves to the gut wall. They have then become post-flagellates, but a fresh supply of nutriment may lead them to develop again into fully-formed flagellates. The terminology, however, is not inconvenient, since the fully-formed flagellates in an insect, whether of the leptomonas, crithidia, or trypanosome form, represent the height of an infection. The rounded or short forms occurring before this stage is reached are found in the stomach and are pre-flagellates, while those developed after it in the hind-gut are post-flagellates.

At certain stages of their development in the invertebrate the flagellates may show a tendency to become attached to the lining cells of the organs (gut, Malpighian tubes, salivary glands) in which they live (Fig. 150, 9-11, 21-23, 40-45). This attachment, which takes place by the flagellar end of the body, is associated with a change in morphology. There is a loss of the flagellum, though the portion of the axoneme between the kinetoplast and the anterior extremity of the body persists. In this condition the flagellates may still retain the trypanosome, crithidia, or leptomonas structure as far as arrangement of the kinetoplast, nucleus, and undulating membrane is concerned. The attached flagellates are usually subject to a shortening of the body, so that every transition between the elongate forms and ovoid leishmania forms may be seen attached to the surface of the cells. On the other hand, the posterior portion of the body of the attached flagellate may undergo an overgrowth (Fig. 150, 12, 45). An extreme type of this condition is seen in the case of the Cercoplasma forms described by Roubaud (1908*a*, 1908*b*, 1911*a*) for *Herpetomonas mirabilis* and *H. mesnili* (Fig. 172). Similar forms were seen by Swingle (1911) in the case of *H. lineata*, in which the post-nuclear part of the body may reach a length of 300 microns. It seems to the writer that the Rhynchoidomonas forms of Patton (1910*a*), in which the axoneme terminates at the anterior end of the body, are probably attachment forms of a flagellate of the trypanosome type (Fig. 150, 45, Fig. 174). In these there is a certain degree of overgrowth of the posterior portion of the body, though nothing comparable with that which occurs in the Cercoplasma

forms noted by Roubaud and Swingle. At any time, probably with the advent of fresh nutriment into the intestine, these attached forms may become free, and, as a result of increase in length of the axoneme, develop flagella for a swimming mode of life.

ORIENTATION AND ORIGIN OF THE DIFFERENT TYPES.

It will be seen that in the simplest flagellate type, the leptomonas, the flagellar end is undoubtedly anterior, for the living organism progresses in this direction, and in the more highly constructed trypanosomes with undulating membranes the flagellar end, according to the view expressed above, is still anterior. In the blood of certain fish there occurs another type of flagellate, the trypanoplasm, which possesses two flagella (Fig. 151). During progression, one is directed forwards and the other, attached to an undulating membrane, backwards. There is little doubt that the anterior end of this organism is the one from which the forwardly directed free flagellum arises, and it has been supposed by some that the trypanosome type has been derived from these forms by the suppression of the free flagellum. It will be evident that, if this is the case, the flagellar end of a trypanosome must be regarded as posterior. The evidence in favour of trypanosomes as they occur in vertebrates having originated from the leptomonas and crithidia forms of invertebrates seems almost conclusive, while the trypanoplasms undoubtedly belong to quite another group of flagellates (see p. 637). Léger, L. (1904*g*), expressed the opinion that some trypanosomes had been derived from a trypanoplasm ancestor (trypanosomes with flagellum posterior), while others had originated from leptomonas ancestors (trypanosomes with flagellum anterior). Lühe (1906)

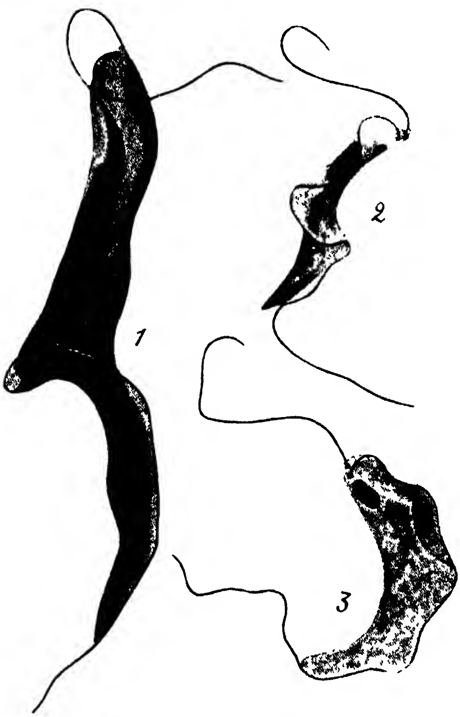


FIG. 151.—TRYPANOPLASMS FROM FISH (× 2,000). (AFTER MINCHIN, 1909.)

1. *Trypanoplasma keysselitzi* of the tench.
2. *Trypanoplasma abramidis* of the bream.
3. *Trypanoplasma borreli* of the rudd.

expressed similar views, and considered the two types of trypanosome generically distinct. Woodcock (1906), in discussing the phylogeny of the trypanosomes, arrived at the conclusion that two distinct families are represented (Trypanosomatidæ and Trypanomorphidæ), one including heteromastigine forms evolved from trypanoplasm ancestors originally parasitic in the vertebrate intestine, and the other including herpetomonadine forms evolved from insect or invertebrate flagellates. In the former the flagellar end is posterior, while in the latter it is anterior. There seems to be, however, no sound argument to support this view. Representatives of both Woodcock's groups are culturable, and in these cultures they all tend to revert to flagellates of the insect type—viz., leishmania, leptomonas, and crithidia forms—a fact which speaks strongly in favour of

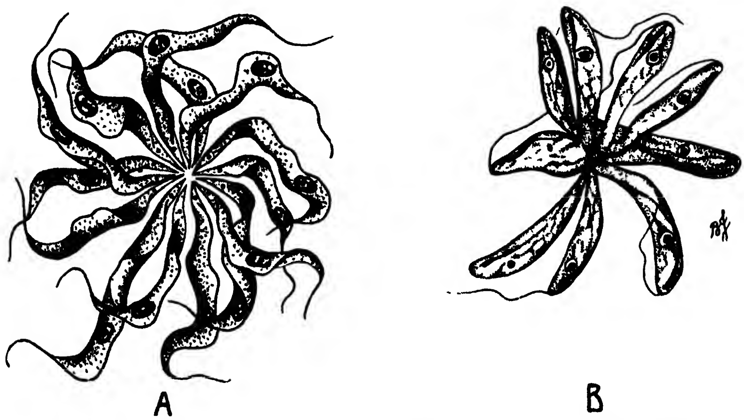


FIG. 152.—AGGLOMERATION OF TRYPANOSOMES AND TRYPANOPLASMS BY THEIR POSTERIOR ENDS AS A RESULT OF THE ACTION OF SERA (× *ca.* 1,300). (A, AFTER LAVERAN AND MESNIL, 1912; B, AFTER SCHINDERA, 1922.)

A. *Trypanosoma lewisi.* B. *Cryptobia helicis.*

the similarity of their origin. In his genus *Trypanomorpha*, Woodcock places *Trypanosoma noctuæ* and probably some of the avian and mammalian trypanosomes, while the genus *Trypanosoma* includes all other forms.

The phenomenon of agglomeration first demonstrated by Laveran and Mesnil (1900*a*) in the case of *Trypanosoma lewisi* may be regarded as throwing some light on this question. When acted upon by certain sera the trypanosomes become clustered in rosettes, with their flagellar or anterior ends directed outwards and their non-flagellar ends united at the centre of the cluster (Fig. 152, A). Schindera (1922) has shown that *Cryptobia helicis* of the snail similarly becomes agglomerated under the influence of sera. In this case, again, it is the anterior end with the free flagellum which is directed outwards, while the posterior end with the posterior flagellum is at the centre (Fig. 152, B).

The most generally accepted view is that the trypanosomes of vertebrates were originally purely insect flagellates which gradually became adapted to the blood medium when the insects became blood-suckers. The flagellates then passed into the vertebrate, and became adapted to life in the blood-stream. Minchin (1908), however, held the opinion that trypanosomes were originally intestinal flagellates of vertebrates which thence passed into the blood-stream, and secondarily became parasites of blood-sucking insects. In a later paper (1914) he appears to have relinquished this view, and writes of the ancestral forms of the trypanosomes as insect flagellates. Mesnil (1918), however, expresses himself in favour of the view that trypanosomes originated from leptomonas forms parasitic in the intestine of the vertebrate. It seems to the writer that the evidence available points to the evolution of trypanosomes from purely insect flagellates, as explained above.

SUBDIVISION INTO GENERA.

The grouping into different genera of the flagellates belonging to the family under consideration is, in our present state of knowledge, exceedingly difficult, and for the want of some definite scheme great confusion in nomenclature has resulted. Some of the flagellates have only an invertebrate host; others have two hosts, a vertebrate and an invertebrate; while others, again, are known only in the vertebrate. According as to whether they are limited to an invertebrate, or have both a vertebrate and an invertebrate host, or whether, in their highest stage of development, they reach the leptomonas, crithidia, or trypanosome form, it is possible to group them in general in the following provisional manner, always remembering that the flagellates which have no vertebrate host, and which pass directly from insect to insect, do so in an encysted stage, which does not occur in those flagellates which pass from insect to vertebrate or *vice versa*. Other flagellates of the leptomonas type have an invertebrate and a plant host.

1. Flagellates of the genus *Leptomonas* are those which never develop beyond the leptomonas stage. In the course of their life-history they show only the leishmania and leptomonas forms. They are confined to invertebrate hosts, and pass from one to another by means of cysts voided with the dejecta.

2. Flagellates of the genus *Crithidia* show, in the course of their development, leishmania, leptomonas, and crithidia forms. They are limited to invertebrate hosts, as in the members of the genus *Leptomonas*, and are transmitted in a similar manner by means of cysts.

3. Flagellates of the genus *Herpetomonas* are, again, purely invertebrate

parasites, but they attain a higher degree of development. In their cycle are found all four types—leishmania, leptomonas, crithidia, and trypanosome. Here, again, infection is passed on by encysted forms.

4. Flagellates of the genus *Leishmania* resemble those of the genus *Leptomonas* in having only the leishmania and leptomonas forms, but they differ in having both a vertebrate and an invertebrate host. Both the leishmania and leptomonas forms may occur in either host. It is presumed

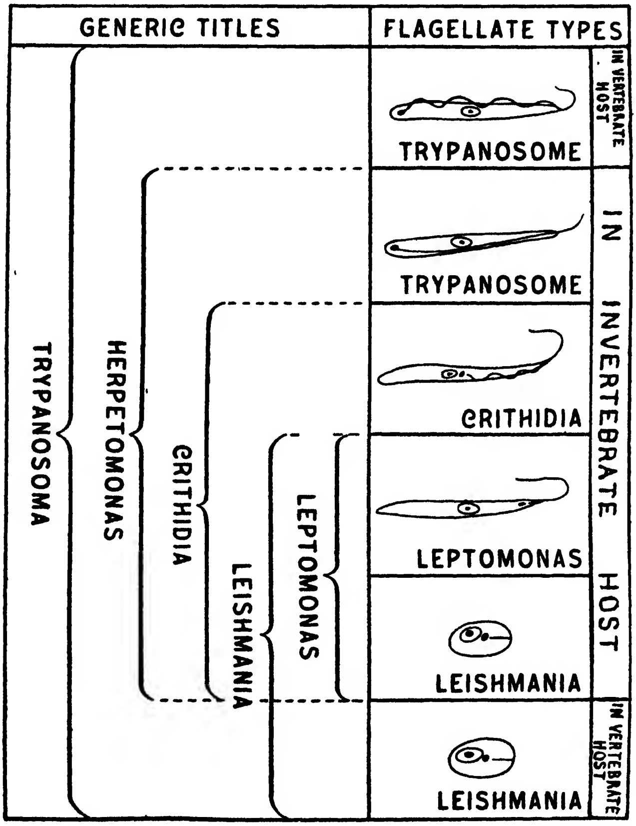


FIG. 153.—DIAGRAM OF CLASSIFICATION OF THE TRYPANOSOMES AND ALLIED FLAGELLATES. (AFTER WENYON, 1913, 1921.)

that infection passes from invertebrate to vertebrate or from vertebrate to invertebrate, but the exact mechanism of this is not yet known.

5. Flagellates of the genus *Phytomonas*, which resemble those of the genus *Leptomonas*, but differ in having both an invertebrate and plant host.

6. Flagellates of the genus *Trypanosoma* have also a vertebrate and invertebrate host. They resemble those of the genus *Herpetomonas* in

having at various stages of their development all four types of flagellate. They are passed from invertebrate to vertebrate or from vertebrate to invertebrate.

It will be seen that to complete the series there should be a form with two hosts corresponding with the genus *Crithidia*, but no such flagellate is known at present.

This grouping of these nearly related organisms in the genera defined above may be represented in tabular form (Fig. 153).

As will be explained below, the type species of the genus *Leptomonas* is a flagellate of the nematode worm *Trilobus gracilis*. Unfortunately, this flagellate has never been re-examined in the light of present-day knowledge, so that there is some doubt as to its true nature. For the present purpose it is regarded as having the structure of the members of the genus as described above. The type species of the genus *Herpetomonas* is *H. muscarum*, a common flagellate of the house-fly. It is assumed here that the trypanosome forms which may occur in association with it are actually stages in its development, and recent work supports this view, though some observers believe they belong to a distinct parasite of flies. If this latter view should prove correct, then a new name would have to be found for the genus *Herpetomonas* as here defined. It would, however, in no way invalidate the scheme of classification. Knowledge concerning many of these flagellates is still very imperfect. It is probable that some of those which are only known in an invertebrate host will ultimately be found to have a vertebrate one also. On the other hand, those which are only known in a vertebrate host have undoubtedly an invertebrate host as well, though it is at present unknown. There is only one exception to this rule in *Trypanosoma equiperdum*, the cause of dourine of horses, which passes from vertebrate to vertebrate without the intermediary of an invertebrate, though the possibility of an alternative method of transmission through an invertebrate cannot be excluded entirely.

The matter is still further complicated by the claims made by some observers that certain of these flagellates, which in nature appear to be purely insect parasites, are experimentally inoculable into vertebrates, and produce in them a condition somewhat resembling that produced by flagellates which naturally have both hosts. It will be realized how closely all the forms are related to one another. Moreover, some of them appear to be actually in a process of transition from one genus to another. This relationship is further illustrated by their behaviour in culture media. For instance, a flagellate which is only known in the trypanosome stage in the blood of a vertebrate will in such a culture develop into a multiplicity of crithidia, leptomonas, and leishmania forms.

Similarly, it was first shown by Rogers (1904) that the leishmania forms of the parasites of kala-azar were in reality flagellates by their development into leptomonas forms in culture.

It is probable that the transformation which occurs in culture is an imitation of that which takes place in the invertebrate host.

In this description, wherever the words leishmania, leptomonas, crithidia, or trypanosome are employed in an adjectival sense, they refer to stages in the development of any of the flagellates which show these particular forms, and are not used in a generic sense. When they are employed as nouns, they refer to a member of the particular genus. Thus one may speak of a leishmania form of a trypanosome, a trypanosome form of a herpetomonas, or a leptomonas form of a leishmania.

CYTOLOGY OF TRYPANOSOMES AND THE ALLIED FLAGELLATES.

Before describing the individual genera, it will be necessary to consider the structure and method of multiplication in greater detail. The whole group shows a marked uniformity of minute structure, though, as will be seen, considerable variation occurs in the actual size and shape of the body, especially amongst the trypanosomes.

CYTOPLASM.—The body is covered by the periplast, and within it the cytoplasm is generally perfectly clear and of a very fine alveolar structure. Vacuoles may be present, especially the one already referred to, which is near the kinetoplast. It has been described as contractile in *Herpetomonas muscarum*, but this is probably incorrect. Occasionally, forms with a highly vacuolated cytoplasm are seen, but these are abnormal or degenerating individuals.

Apart from the periplast, the cytoplasm is of uniform consistency, and shows no differentiation into ectoplasm and endoplasm. Immediately below the periplast longitudinal fibres can sometimes be made out, especially in some of the larger trypanosomes (Fig. 28, B). These are generally supposed to be myonemes or contractile fibres. Minchin (1909, 1909*a*), who had observed them in the trypanosomes of the perch and eel, was unable to distinguish them in the smaller *Trypanosoma lewisi*.

GRANULES.—Various kinds of granule may be present in the cytoplasm, especially anterior to the nucleus. Some of these may take a red or purple tint with Romanowsky staining. In *T. lewisi*, Minchin (1909*a*) refers to them as "chromatoid granules," and many writers have considered them to be volutin. On account of their affinity for certain chromatin stains, they have sometimes been mistaken for nuclei.

Flagellates of this group are sometimes packed with these granules, but their presence seems to depend upon the rate of metabolism. The

FIG. 154.—VARIOUS INTERNAL STRUCTURES WHICH HAVE BEEN DESCRIBED IN TRYPANOSOMIDÆ. (1-5, AFTER CHATTON AND LEGER, 1911; 6, AFTER MCCULLOCH, 1915; 7, AFTER PROWAZEK, 1904; 8, AFTER MINCHIN, 1909; 9-10, AFTER NIESCHULZ, 1922.)

[*For description see opposite page.*

flagellates which occur in cultures of trypanosomes and leishmania are sometimes perfectly free from granules of this kind, while at other times many are present. Their exact nature is doubtful, but there is no evidence that they have originated from the chromatin of the nucleus, as some have supposed. Doflein (1910) has noted the presence of fat globules in the flagellates occurring in old cultures of *Trypanosoma rotatorium* of the frog. In *Herpetomonas muscarum* in the posterior region of the body there sometimes occur rod-shaped structures which show bipolar staining (Fig. 159). The writer (1913*a*) regarded them as bacteria which had entered the cytoplasm, but similar structures in other flagellates have been interpreted as evidence of a process of internal budding (see p. 338).

Granules other than metaplastic ones which have arisen as a result of metabolism have been occasionally described in the cytoplasm. Thus, in *Trypanosoma raiæ* (Fig. 247, 12, 13) a granule surrounded by a clear area and lying near the nucleus was described by Robertson (1909*a*). Minchin (1909*a*) described a refringent granule lying behind the kinetoplast in *Trypanosoma lewisi*. It was present in specimens killed by osmic acid vapour and examined wet without further treatment, but was not detected in stained specimens (Fig. 154, 8). The writer recently noted that in an ordinary dried and stained film of *T. lewisi* nearly every trypanosome possessed a fairly deeply staining granule surrounded by a clear halo (Fig. 197, 16). It was of uniform appearance and adjacent to the nucleus. No fibre or filament could be detected in connection with it, and no suggestion can be offered as to its nature or function. In some individuals it was rod-shaped and in others double. A similar rod-shaped body was described by Nieschulz (1922*a*) in the cultural forms of bird trypanosomes (Fig. 154, 10).

AXIAL AND OTHER FILAMENTS.—Another structure which has been described is the axial filament. Prowazek (1905) depicted a complicated fibrillar system in *T. lewisi*. A filament connected the karyosome of the nucleus with the parabasal body, from which another filament ran through the cytoplasm to another granule situated posteriorly to the nucleus, while from it another passed to the anterior end of the body. None of these structures were detected by Minchin (1909*a*) in his careful study of the cytology of *T. lewisi*. Prowazek (1904) described an axial filament in *Herpetomonas muscarum* as extending from a centrosome associated with

1-5. *Herpetomonas drosophilæ* in division, showing formation of axoplast from the dividing kinetoplast (× *ca.* 4,000).
6. *Crithidia leptocoridis*, showing complicated system of fibrils (× 3,500).
7. *Herpetomonas muscarum*, showing fibrils (× *ca.* 4,000).
8. *Trypanosoma lewisi*, showing refractile granule, which is present in wet osmic killed trypanosomes (× 3,000).
9-10. Cultural forms of bird trypanosome, showing granule within nuclear membrane and rod-shaped structure in the cytoplasm (× 3,000).

the kinetoplast to another centrosomic body near the posterior end of the flagellate (Fig. 154, 7). The writer (1913*a*) was unable to detect such a filament in this flagellate. A similar filament (axoplast) was described by Chatton and Leger, M. (1911), in *Leptomonas* (*Herpetomonas*) *drosophilæ* (Fig. 154, 1-5). It extended from the kinetoplast to the posterior end of the body. When division was taking place it degenerated, and a new one was formed between the two daughter kinetoplasts. At first straight, it soon became U-shaped. The limbs of the U increased in length till finally each was as long as the body, which meanwhile had commenced dividing from its anterior end. When division of the body was complete, the limbs of the U formed the new "axoplasts" of the daughter flagellates. In *Crithidia leptocoridis* parasitic in the gut of the box-elder bug, McCulloch (1915) has described a system of fibres still more complicated (Fig. 154, 6). In this case the "axostyle" commencing in the blepharoplast runs to the posterior end of the body, where it terminates in a granule called the "chromatin granule." In addition, there is a fibre connecting the blepharoplast or the kinetoplast with the karyosome of the nucleus, and another ("myoneme") running from the posterior end of the body to terminate in the flagellum. In a subsequent work, however, the same writer (1919) gives a diagram of *C. leptocoridis* which only shows one of these fibres—namely, that connecting the blepharoplast with the karyosome of the nucleus. The majority of observers have not detected or described these fibres in the flagellates they have examined. The writer (1913*a*) has examined many forms which have been carefully fixed and stained, and has seen no such structures present with the constancy which would be expected if they were essential parts of the anatomy. If large numbers of individuals of any species are examined, occasionally fibres resembling those described by the various writers may be seen, but other explanations of their presence can be given. Folds or creases in the periplast or abnormally developed flagella may give rise to these appearances. The whole group is such a homogeneous one that it is highly improbable that structures so complicated would be present in one species and completely absent in another.

A fibre connecting the blepharoplast or parabasal with the karyosome of the nucleus has been more frequently described. McCulloch (1915) refers to it as the rhizoplast, and not only mentions its occurrence in *Crithidia leptocoridis* (Fig. 154, 6), but also (1917) in *C. euryophthalmi* (Fig. 168), while Kofoid and McCulloch (1916) note it in *Trypanosoma* (*Herpetomonas*) *triatomæ*, a flagellate of the bug *Neotoma fuscipes*, Chagas (1909) described a similar connecting fibril in the case of the developmental forms of *Trypanosoma cruzi* in the bug *Triatoma megista*.

Here, again, it may be said that this fibre has not been seen with any

constancy, and, if present, it has been overlooked by the majority of competent observers. The complicated system of fibres associated with the nuclear changes and divisions of *Trypanosoma noctuæ* and its development in the owl and mosquito, as described by Schaudinn (1904), are only of historic interest.

An axial filament was described in *Herpetomonas muscarum* from the fly *Calliphora erythrocephala* by Alexeieff (1911*e*). He also observed in this fly the peculiar Rhynchoidomonas forms first described by Patton

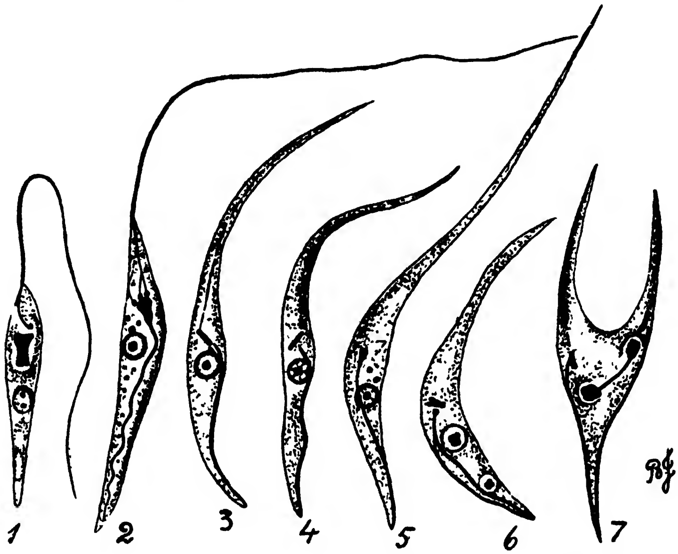


FIG. 155.—*Herpetomonas muscarum* FROM INTESTINE OF *Calliphora erythrocephala* (× 1,500), ARRANGED TO SHOW ALEXEIEFF'S VIEW OF THE ORIENTATION OF THE RHYNCHOIDOMONAS FORMS. (AFTER ALEXEIEFF, 1911.)

1. Typical leptomonas. 2. Leptomonas with rhizostyle.
3-4. Axoneme of flagellum still visible, while rhizostyle is more marked.
5-6. Axoneme no longer visible, while rhizostyle is well developed.
7. Dividing form.

It appears probable, however, that the forms at 3-7 should be reversed, and that the structure called the rhizostyle is in reality the attached axoneme, which is longer owing to the backward migration of the kinetoplast.

(1910*a*) in *Lucilia* (Fig. 155). In the latter there is a deeply staining line running from the kinetoplast along the surface of the body past the nucleus to the pointed extremity of the body. The other extremity is drawn out into a tapering process. In some of these forms there is to be detected a faintly staining line extending from the kinetoplast to the end of this process. The natural interpretation of this appearance would be that the deeply staining band is the axoneme, and that the flagellates have the trypanosome structure, though a somewhat remarkable one. Alexeieff, however, interprets them differently.

He regards the deeply staining band as the axial filament, here well developed, while the faintly staining line passing to the opposite extremity, and not always present, as representing the axoneme. Of the two views, it seems to the writer that the first one is correct, otherwise one must assume that this particular form is totally different from all other flagellates of the group. The writer (1913*a*) studied these forms in the house-fly, and could find no evidence to support Alexeieff's view.

NUCLEUS.—The nucleus, which can only be satisfactorily studied in specimens which have been prepared without drying, generally consists of a nuclear membrane enclosing a clear space, at the centre of which is a karyosome which stains deeply with chromatin stains. In some of the larger trypanosomes, radiating fibres—the nuclear meshwork—can be seen connecting the karyosome with the nuclear membrane (Fig. 247). The latter is clearly seen as a definite structure in the largest trypanosomes. In the smaller forms it may be difficult to distinguish from the surrounding cytoplasm, the nucleus appearing as a clear space in the cytoplasm with the karyosome at its centre. In such cases it is probably safe to assume that a very fine membrane is present, for the clear space persists in nuclear division, during which it becomes elongated and finally divided. In the nuclei of the larger trypanosomes, in addition to the central karyosome, smaller granules, apparently of a chromatin nature, are sometimes present on the inner surface of the nuclear membrane or even distributed upon the nuclear meshwork. Occasionally, in place of the single karyosome, several comparatively large chromatic bodies are present. The karyosome usually stains uniformly and intensely, but in the larger forms several more deeply staining areas may be present, suggesting, as Robertson (1909*a*) has pointed out for *Trypanosoma raiæ*, that it may be made up of two substances, the chromatin proper and a plastin material. Sometimes a more deeply staining granule has been distinguished at the centre of the karyosome, and from what occurs in nuclear division this granule has been interpreted as a centriole or intranuclear centrosome. Nieschulz (1922*a*) has noted the presence of a small granule on the nuclear membrane of the crithidia forms which appear in cultures of bird trypanosomes (Fig. 154, 9). It was not present in the trypanosome forms. It must be remembered, however, that the appearance of the karyosome after such stains as iron hæmatoxylin varies considerably with the degree of extraction of the stain.

In nuclear division, as usually seen in wet fixed films, there is an elongation of the nucleus associated with an elongation of the karyosome (Fig. 156, 1). The latter finally becomes constricted at its centre and divided into two daughter karyosomes. The nuclear membrane then becomes constricted between these, and two nuclei similar to the original one are produced.

The division is generally equal, so that the two daughter nuclei are approximately of the same size. Not infrequently during division the nucleus becomes much elongated, while the halves of the divided karyosome occupy the poles and are connected by a fine line, which is usually referred to as the centrodesmose, and is supposed to connect the two daughter centrioles which may be presumed to lie embedded in the daughter karyosomes. This is all the more striking in cases which have sometimes been noted where the chromatin of the nucleus, instead of being concentrated into a single karyosome, is in the form of two or more granules lying on the inner surface of the nuclear membrane. At nuclear division, a small central granule first divides, and the two separating halves are connected by a fibre. The chromatin granules themselves then divide into two groups, and two daughter nuclei are ultimately formed. In these cases the central granule, which may or may not be a true centrosome, is not obscured by concentration of the chromatin around it in the form of a karyosome. In other cases it would seem that the central granule divides, and the halves, still connected by a fibre, separate, and eventually pass out of the karyosome, the division of which is retarded. There is then produced an elongated nucleus with a granule at each end, and a centrodesmose connecting them. The karyosome, still undivided, lies at the centre of the centrodesmose. Though the gradual elongation and constriction of a uniformly staining karyosome is the usual appearance in nuclear division, a number of observers have maintained that after appropriate staining the elongating karyosome can be resolved into a spindle, upon the fibres of which the chromatin is distributed in the form of granules or chromosomes. Schaudinn (1904) described a mitotic division of the nucleus in *Trypanosoma noctuæ;* Rosenbusch (1908–09) in *T. lewisi* and *T. brucei;* Hindle (1909) in *T. dimorphon;* Chagas (1909) in *T. cruzi;* Alexeieff (1912*e*) in *T. lewisi* and *T. brucei;* Kuhn and Schuckmann (1912), Kuczynski (1917), and Schuurmans-Stekhoven (1919) in *T. brucei;* Hartmann and Nöller (1918) in *T. theileri;* and Nieschulz (1922*a*) in bird trypanosomes. Hartmann and Nöller state that in the case of *T. theileri* there is formed an intranuclear spindle which appears to originate in the achromatic material of the karyosome, while the chromatin substance in the form of granules is first distributed irregularly on the spindle (Fig. 156, 5–8). The spindle elongates, as also does the nuclear membrane, so that there results a long oval nucleus enclosing a sharp-pointed spindle, the ends of which may rest on the nuclear membrane. At this stage the chromatin granules collect at the equator of the spindle to form an equatorial plate, which quickly divides into daughter plates. The latter move towards the poles of the spindle, which divides at its centre. Each half, with the chromatin granules of the daughter plate, which have again become irregularly dis-

tributed on the spindle, concentrates into the daughter karyosome. Nuclear division is completed by the division of the nuclear membrane. During the whole of this process the nuclear membrane has persisted and enclosed the spindle. Nieschulz (1922*a*) has described a similar method

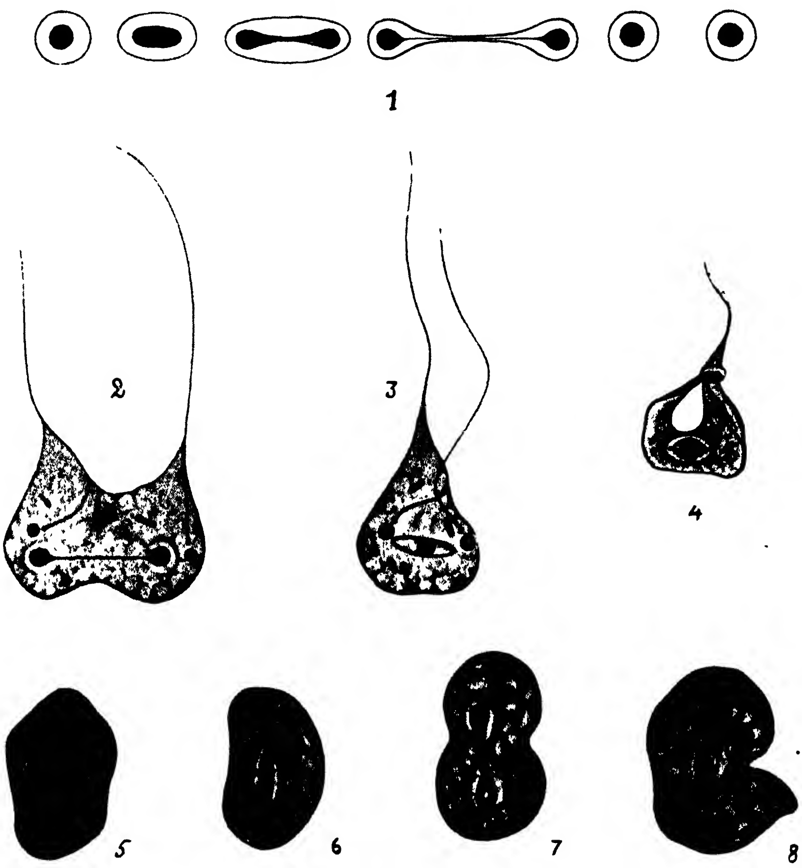


FIG. 156.—NUCLEAR DIVISION IN TRYPANOSOMIDÆ. (1, ORIGINAL; 2-3, AFTER NIESCHULZ, 1922; 4, AFTER CHAGAS, 1909; 5-8, AFTER HARTMANN AND NOLLER, 1918.)

1. Usual appearance in wet fixed films stained by iron hæmatoxylin.
2-3. Two stages in division of the cultural form of the trypanosome of the ring-ousel (*Turdus torquatus*), showing mitosis of the nucleus (× 3,000).
4. Division of the nucleus of *Trypanosoma cruzi* by mitosis (× *ca.* 3,000).
5-8. Division of cultural forms of *Trypanosoma theileri*, showing mitotic division of the nuclei (× *ca.* 2,600).

of division of the nuclei of cultural forms of bird trypanosomes (Fig. 156, 2-4). He noted the formation of an equatorial plate which divided to form daughter plates, but was unable to distinguish individual chromosomes, the plates appearing as dark bands across the spindle. Schulz

(1924) has found that the nucleus of *Leishmania donovani* divides by mitosis, as also that of *Leptomonas fasciculata* in cultures from the intestine of *Culex pipiens* and *Theobaldia annulata*. It seems probable, therefore, that the nucleus of the trypanosomes and the allied flagellates divides by a form of mitosis, but the process is difficult to detect in such minute objects, and can only be demonstrated by special staining. The ordinary appearance at nuclear division is that of an elongating, deeply staining karyosome which becomes constricted at the centre, as described above.

An unusual type of nucleus has been described in the broad, leaf-like forms of *Trypanosoma rotatorium* (Fig. 150, 38). It was first noted by França and Athias (1906) in the trypanosome of *Hyla*, and then by Martin (1907) in *T. boueti*. It was subsequently observed by other writers in *T. rotatorium* of frogs (see p. 592). Instead of being spherical, the nucleus is in the form of a long spindle, which is frequently curved. One end is near the kinetoplast, while the other may be near the posterior extremity of the flagellate. It has been shown by Nöller (1913*b*) that the large trypanosomes of this type in the frog are the survivors of a tadpole infection with flagellates of the more normal type, and are to be regarded as abnormal or overgrowth forms. The long drawn-out nucleus may represent a division process which has been arrested at this stage.

KINETOPLAST.—The kinetoplast consists of the blepharoplast and parabasal body. The two appear to be united. This union has been described as a system of fibres forming a cone, with the parabasal at its base and the blepharoplast at the apex, or as a definite membrane enclosing a space with the parabasal and blepharoplast at opposite poles. That there is such a membrane is borne out by certain appearances seen in degenerating trypanosomes, which the writer (1913*a*) studied in smears made from heavily infected animals some hours after death. It was noted that many trypanosomes were in various stages of disintegration, and that frequently the cytoplasm had disappeared, leaving only the nucleus and the kinetoplast, with the axoneme and flagellum still attached (Fig. 157). The axoneme will be seen to terminate in the blepharoplast, which appears to be lying on the surface of a membrane connecting it with the parabasal. If the parabasal were a free and independent structure, it would be expected that in disintegration or cytolysis the parabasals would not remain united, as they appear to do. Dividing or already divided blepharoplasts and parabasals still show this connection with one another and with the axoneme of the flagellum. It is interesting to note that in these degenerating forms there is no evidence of a fibre connecting the kinetoplast with the karyosome of the nucleus, as several observers have described.

The first indications of division are seen in the kinetoplast. The

blepharoplast becomes transversely elongated, narrowed at its centre, and finally divided into two parts, the axoneme remaining attached to one half. Coincidently with this the parabasal, if a rounded body, also becomes transversely elongated, constricted, and divided into two, and finally two kinetoplasts are produced, each constituted as the original one. In flagellates like *Herptomonas muscarum*, the kinetoplast is an elongated structure with the blepharoplast at its anterior end and the parabasal at its centre. The blepharoplast first divides, and the two halves separate (Fig. 158). The parabasal becomes dumb-bell shaped and also divides. At a certain stage of the process the dividing kinetoplast is in the form of a Y, with a blepharoplast at the end of each of the two anteriorly

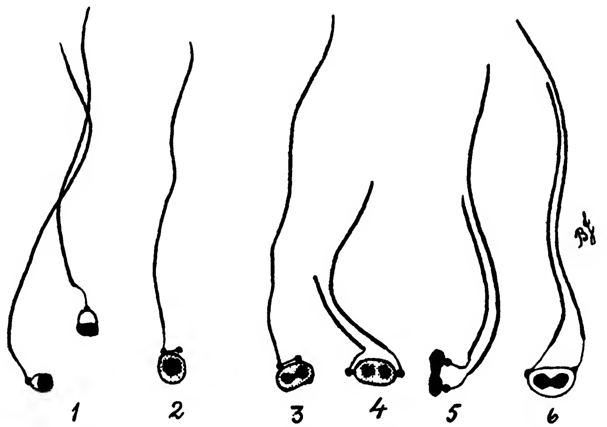


FIG. 157.—KINETOPLASTS AND ATTACHED AXONEMES OF DEGENERATE *Trypanosoma rhodesiense*, AS SEEN IN DRIED FILMS STAINED BY LEISHMAN STAIN. (AFTER WENYON, 1913.)

1. Blepharoplast united to parabasal; the part of the axoneme which borders the membrane appears thicker than the intracytoplasmic portion, owing to a sheath of cytoplasm.
2. Early division with dividing blepharoplast.
3. Division of blepharoplast and parabasal.
4. Completed division of parabasal and blepharoplast and outgrowth of new axoneme.

5 and 6. New axonemes forming before division of parabasal is complete.

directed limbs. The various stages of this division process give the impression that the blepharoplast is leading the way in division, and it appears as if the stress exerted by the separating daughter blepharoplasts causes the parabasal and the entire kinetoplast to divide. The writer (1913*a*) compared this division with that of the nucleus of *Cercomonas longicauda*, which in the resting condition is composed of a spherical nuclear membrane with large central karyosome. The anterior part of the membrane is really cone-shaped, and at the apex of the cone is the blepharoplast, from which two flagella arise. In nuclear division the blepharoplast first divides. As the two halves separate a spindle

is formed between them, while the karyosome breaks up into chromosomes, which arrange themselves upon the spindle as an equatorial plate. In this case the blepharoplast is undoubtedly functioning as a true centrosome. In many respects the division of the nucleus of *Cercomonas longicauda*, as also that of *Heteromita uncinata*, resembles that of the kinetoplast of *Herpetomonas muscarum*, in which, however, the parabasal does not form chromosomes, but merely splits into two equal parts (Figs. 68 and 259).

Robertson (1913) has shown that in *Trypanosoma gambiense* division of the kinetoplast may take place in such a manner that the two daughter blepharoplasts are connected by a fibre, at the central point of which the parabasal is situated. When the daughter blepharoplasts still further separate, the parabasal divides and two kinetoplasts are thus formed. Such a method of division has a striking resemblance to that frequently seen

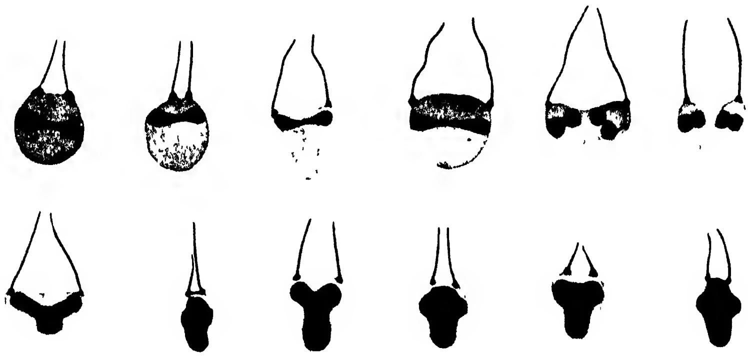


FIG. 158.—DIVIDING KINETOPLASTS OF *Herpetomonas muscarum*. (AFTER WENYON, 1913.)

Upper row after fixation in Hermann's fluid.
Lower row after fixation in Schaudinn's fluid.

in division of the nucleus itself. This raises the question of the nuclear nature of the kinetoplast. Hartmann (1907), assuming this to be the case, regards the flagellates of this group as possessing two nuclei, and places them in a special order, the Binucleata. The evidence of the true nuclear nature of the kinetoplast in the sense that it is a second nucleus is, however, wanting. The mitotic divisions of the kinetoplast with formation of chromosomes, which some observers have recorded, can hardly be taken seriously.

If, however, such a mitotic division actually occurred, it would be strong presumptive evidence of the nuclear nature of the kinetoplast. From analogy with other flagellates, the terminology here employed appears to be the safest one. In many flagellates, axonemes arise from a blepharoplast in close association with which is a parabasal body which

assumes various shapes and forms. In division, both the blepharoplast and parabasal body divide (Fig. 32).

Schaudinn (1904), in his description of what he regarded as the origin of the "binucleate" trypanosome from the uninucleate halteridium of the little owl, supposed the kinetoplast to arise by an unequal division (heteropolar mitosis) of the nucleus, the smaller portion becoming the kinetoplast. The origin of the flagellar blepharoplast from the nucleus was described by Jameson (1914) in *Parapolytoma satura*, and by Entz (1913, 1918) in *Polytoma uvella* (see p. 52). Though there may be some evidence that in other flagellates the blepharoplast arises by division of an intranuclear centrosome (*P. uvella*), the proof that the flagellates of the trypanosome group have a stage devoid of a kinetoplast is still wanting. At certain stages of development of these flagellates, especially when they assume the leishmania form, the kinetoplast approximates to the nucleus to such an extent that the two frequently lie in complete apposition, and by some observers they have been supposed to fuse. Roubaud (1911*b*, 1911*c*) described such a fusion in the leishmania forms of *Herpetomonas* (*Leptomonas*) *sudanensis*, a flagellate of an African fly (*Pycnosoma*), but that actual union had taken place seems very doubtful. In this connection, mention may be made of the remarkable process of union of kinetoplast and nucleus, which was first described by Moore and Breinl (1907) for *Trypanosoma gambiense*, and later by the same observers (1908) for *T. equiperdum*; by Moore, Breinl, and Hindle (1908) for *T. lewisi*; and by Fantham (1911) for *T. gambiense* and *T. rhodesiense*. The process is described as taking place in the blood and organs of heavily infected experimental animals. A periodic variation in the number of trypanosomes in the blood occurs, and at the height of the wave there appear in the blood forms which possess an axial filament extending from the kinetoplast to the nucleus. The filament, which is supposed to consist of chromatin material, breaks up into granules which fuse with the nucleus. The cytoplasm of the trypanosome is then cast off, leaving a small rounded body ("latent body"), consisting of little more than the nucleus with which the chromatin of the kinetoplast is supposed to have united. The "latent bodies" are to be found in the smears of the lung, spleen, and other organs. They are said to give rise to trypanosomes again by formation of cytoplasm around them, while by division of the intranuclear centrosome, one half of which separates, the new kinetoplast is formed. In the work of Moore, Breinl, and Hindle, the transformation of the trypanosome into the "latent body," and the converse process of the growth of the latter into the trypanosome, was studied in stained films in which a series of forms supposed to illustrate the process were depicted. That leishmania forms do actually occur during the course of development of many trypanosomes, and that

these may again grow into trypanosomes, there is no question, but it must not be forgotten that in the body of heavily infected animals many trypanosomes undergo degenerative changes. It seems to the writer that the whole process as described by Moore and Breinl is the result of the combination of these involution forms in a hypothetical cycle. Fantham (1911), however, maintains that he actually observed the growth of these latent bodies *in vitro*, and gives a figure purporting to show the transformation of a small "latent body" of the leishmania type into a fully-formed trypanosome possessing flagellum and undulating membrane. The resulting organism was many times the bulk of the original body, and the whole of this remarkable metamorphosis is stated to have taken place in about one hour. It is known that the leishmania forms of the parasites of oriental sore, or kala-azar, require at least forty-eight hours to become fully-formed flagellates, so that it is impossible to accept the statement regarding such a rapid development without confirmation.

It seems, therefore, that no reliable evidence of the origin of the kinetoplast of trypanosomes and the allied flagellates from the nucleus has yet been produced, and though in some respects the kinetoplast behaves like a nucleus during division, this does not justify an assertion that it is a true nucleus. The whole question is a very difficult one, and involves the more general one of the definition of the nucleus itself.

FLAGELLUM.—The flagellum arises from the anterior end of the body, and, like that of other flagellates, consists of a cytoplasmic sheath enclosing an axial filament, the axoneme, which can be traced through the cytoplasm to the blepharoplast. In flagellates of the crithidia and trypanosome type the axoneme passes to one side of the body, and then along the surface of the body or on the edge of a ridge of cytoplasm—the undulating membrane—to enter the flagellum at the anterior end of the flagellate. The simplest arrangement occurs in organisms of the leptomonas type. It can be seen that the axoneme is much finer than the flagellum, and this is an indication that the flagellum is composed of an axial filament, the actual continuation of the axoneme, and a sheath of the periplast. In the degenerated and cytolized trypanosomes already referred to (Fig. 157), it will be noted that the flagellum is still attached to the blepharoplast by a very fine line which represents the intracytoplasmic portion of the axoneme.

Furthermore, after division of the blepharoplast, the half which has no attached axoneme commences to form a new one as an outgrowth, which grows parallel to the original axoneme and close to it. When the new axoneme reaches the surface of the body, it may continue its growth within the sheath of the old flagellum, so that when the sheath divides longitudinally, an appearance of longitudinal division of the flagellum

may result. As regards the origin of the new flagellum, there has been some difference of opinion. Some observers, as, for instance, Laveran and Mesnil (1904, 1912), Minchin (1908), Woodcock (1909), and others, have described longitudinal splitting of the flagellum, but the writer (1913*a*), from observations on *Herpetomonas muscarum* and trypanosomes, came to the conclusion that a longitudinal division never occurs, and that the new flagellum is the result of the growth of a new axoneme from the newly-formed blepharoplast, a view previously upheld by Schaudinn (1904), MacNeal (1904), and Prowazek (1905). Hartmann and Nöller (1918), in a study of the cytology of *Trypanosoma theileri*, arrive at the same con-

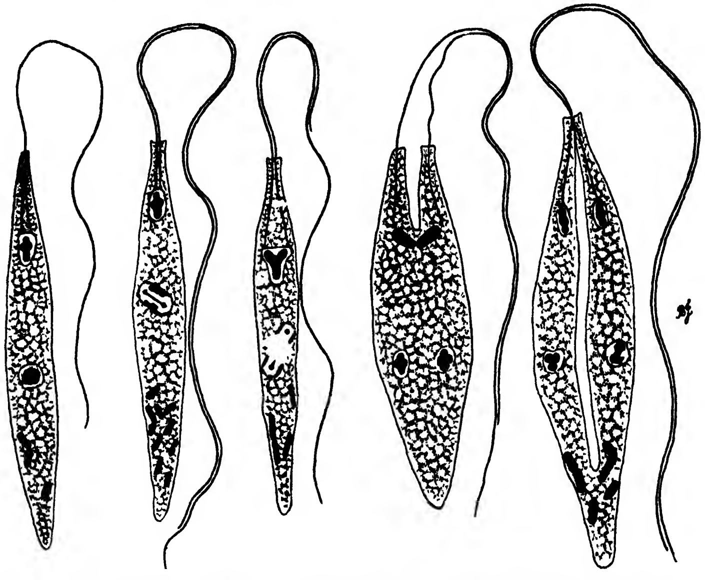


FIG. 159.—DIVIDING FORMS OF *Herpetomonas muscarum* (× 2,000). (AFTER WENYON, 1913.)

The outgrowth of a new axoneme from the divided blepharoplast produces the appearance of an organism with two flagella.

clusion, as also did Rosenbusch (1909*a*), though he gave an erroneous description of the division of the kinetoplast. Mackinnon (1910), in the case of *H. homolomyiæ* and *H. scatophagæ*, describes the intracytoplasmic portion of the axoneme as dividing, the new flagellum being then formed as an outgrowth of the new axoneme. There seems little doubt that the flagellum is entirely formed as a result of the outgrowth of a new axoneme from the new blepharoplast, a process which may even commence before division of the blepharoplast is complete. In some cases the new flagellum attains a considerable length before the kinetoplast is actually divided, so that the organisms appear to have two flagella. *H. muscarum*

is a case in point, for in certain infections this precocious flagellum formation may have taken place to such an extent that nearly every individual has two flagella (Fig. 159). It was this appearance which led Prowazek (1904) to regard this organism as a biflagellate, and to state that the type species of the genus *Herpetomonas* necessarily possessed two flagella. It was proved, however, by the work of Patton (1908*b*), Porter (1909*b*), Mackinnon (1910), and the writer (1911*a*) that the forms with two flagella were in reality dividing forms in which precocious flagellum formation had taken place in anticipation of coming division of the flagellate.

França (1920*a*) maintains that in *H. muscarum* there is actually a division of the kinetoplast, intracytoplasmic portion of the axoneme (rhizoplast), and the flagellum, while in flagellates of the genus *Leptomonas*, after division of the kinetoplast, a new axoneme grows out from the daughter blepharoplast to form a new rhizoplast and flagellum. It appears to the writer that such a distinction cannot be drawn between the genera *Herpetomonas* and *Leptomonas*.

UNDULATING MEMBRANE.—As already explained, in the crithidia and trypanosome forms the axoneme passes along the surface of the body to its anterior end, where it may or may not be continued into a flagellum. In the majority of crithidia and trypanosomes the line of attachment of the axoneme is raised into a thin ridge, the undulating membrane, which varies in length with the position of the kinetoplast. It is attached to the convex edge of the curved, blade-like body of the flagellate. The free border is longer than the attached one, hence it is thrown into folds. The axoneme runs along the free border, and the constant undulating movements of the membrane are probably the result of contractions of the axoneme, though some observers believe that they are due to certain myoneme fibres which they claim to have detected. The membrane consists of little more than the periplast, while the axoneme runs in a canal at its margin. When the axoneme leaves the body of the flagellate, the periplast is continued as the sheath of the flagellum. In some trypanosomes the undulating membrane is broad and well developed (*Trypanosoma rotatorium*), while in others it is very narrow (*T. congolense*). On the other hand, in the trypanosome stages which occur in the development of purely insect flagellates (*Herpetomonas*), well-developed undulating membranes may be present in some cases, while in others there is no definite membrane, the axoneme merely passing along the surface of the body (Fig. 150, 40–45). This condition is well seen in the trypanosome stages of *H. mirabilis* (Fig. 172).

METHOD OF REPRODUCTION.

Reproduction takes place most usually by longitudinal fission (Fig. 160). After division of the kinetoplast and nucleus, and formation of a new flagellum, as described above, the cytoplasm divides by a fission commencing at the anterior end between the flagella and extending backwards till two flagellates result. The resulting organisms remain attached by their posterior ends for some time till final separation takes place. The newly-formed flagellates are usually equal in size, but quite frequently they are unequal, so that a small form may be separated from one many times its size. In these cases, though the cytoplasm of the two daughter forms may differ in amount, the nuclei and kinetoplasts are equally divided. In the crithidia and trypanosome forms the division is more complicated, owing to the presence of the undulating membrane (Fig. 160, 9-16). In both these, after division of the blepharoplast has commenced, a new axoneme begins to form from what will be the daughter blepharoplast. This grows parallel to the original axoneme along the border of the undulating membrane, and as the extremity of the new axoneme is closely applied to the original one, the impression of longitudinal splitting may be given. After the new axoneme has grown to some extent, the undulating membrane commences to split from behind forwards and between the two axonemes. The point up to which the membrane is split at any stage of the process is always a little behind the end of the new axoneme, so that beyond the point to which the membrane has split the new and old axonemes still lie close together. The stage at which complete separation of the axonemes takes place or the extent of division of the membrane depends on the size of the daughter individual. If division into two equal flagellates is taking place, then the process of new axoneme formation and splitting of the membrane extends right up to the anterior end of the flagellate. If, however, the division is into unequal flagellates, then there is a shorter axoneme formed and a shorter membrane is split off. In either case there results a flagellate with two axonemes, flagella, and undulating membranes, and as division of the kinetoplast and nucleus will then be complete, with two kinetoplasts and two nuclei. At this stage the body of the flagellate divides, the fission commencing at the anterior end between the two flagella and membranes. It extends backwards till two flagellates are formed, each with a nucleus, kinetoplast, undulating membrane, and flagellum. In the division of a trypanosome, therefore, the splitting of the membrane and growth of the axoneme takes place from behind forwards, while the body divides from before backwards (Fig. 160, 13–16). Division of the non-flagellate leishmania forms also takes place after division of the kinetoplast and nucleus. In these cases, in which the axoneme is still

present, it will be seen that at division a new axoneme is formed from the blepharoplast, but it does not extend beyond the surface of the body into

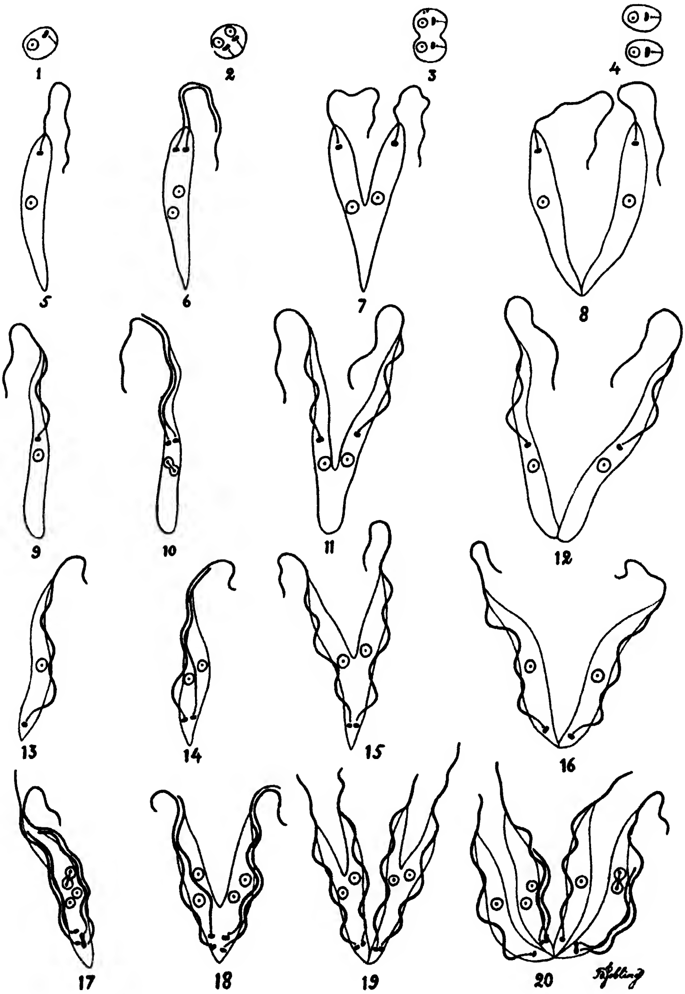


FIG. 160.—DIAGRAMMATIC REPRESENTATION OF METHOD OF DIVISION OF THE VARIOUS FORMS OF THE TRYPANOSOMIDÆ. (ORIGINAL.)

1-4. Division of leishmania form. 5-8. Division of leptomonas form.
9-12. Division of crithidia form. 13-16. Division of trypanosome form.
17-20. Delayed division of cytoplasm resulting in appearance of multiple division of trypanosome form.

a flagellum. The more or less rounded body then divides between the axonemes.

It not infrequently happens that, after division of the kinetoplast

and nucleus, and new flagellum formation, the division of the body is delayed for some reason (Fig. 160, 17–20). The daughter kinetoplasts and nuclei may proceed to a further division, giving rise to forms with four sets of these structures. Such forms are sometimes seen in actively multiplying trypanosomes like *T. brucei* in the blood of a rat, where individuals with four nuclei, four kinetoplasts, and four undulating membranes and flagella arise. By a further division of one or more of the nuclei and kinetoplasts, still more complicated forms are produced.

FIG. 161.—*Crithidia euryophthalmi* FROM INTESTINE OF THE BUG, *Euryophthalmus convinus* (× *ca.* 2,400). (AFTER MCCULLOCH, 1919.)

Multiple division forms (spheres) in the epithelial cells of the crop.

FIG. 162.—*Crithidia euryophthalmi* FROM INTESTINE OF THE BUG, *Euryophthalmus convinus* (× 3,500). (AFTER MCCULLOCH, 1919.)

Forms showing what is described as internal budding.

During the development of *T. lewisi* in the flea, the intracellular phase results in the formation of multinucleate forms, in which as many as sixteen trypanosomes are represented before cytoplasmic division occurs (Fig. 200, 5–11). A similar form has been described by McCulloch (1917) as an intracellular phase of the development of *Crithidia euryophthalmi* in the crop of the bug, *Euryophthalmus convivus* (Fig. 161). Multiple segmentation forms of *T. lewisi* also occur in the blood of the rat in the early phases of an infection (Fig. 197). The process has been

described for *Leishmania donovani* by Mackie (1915*a*), and for *T. cruzi* by Hartmann (1917), but in both these cases the descriptions are not convincing, for it would appear from the figures produced that the so-called schizonts are merely the broken-off portions of the cytoplasm of large cells enclosing leishmania which, as a result of degeneration or feeble staining, have their outlines imperfectly defined, so that the appearance of cytoplasmic bodies containing many pairs of nuclei and kinetoplasts is produced. It must be remembered that simple binary fission is the normal method of reproduction, and the multiple segmentation often termed schizogony merely represents retarded division of the cytoplasm, and is not to be compared with the true schizogony which occurs in the Sporozoa as the normal method of multiplication.

Mention must be made of another type of reproduction which has been described in only one instance. This is the so-called internal budding noted by McCulloch (1919) as occurring in *Crithidia euryophthalmi* (Figs. 162 and 168). In this process, repeated division of the nucleus is supposed to take place till the posterior region of the body contains a varying number of separate nuclei. Around each a portion of cytoplasm becomes concentrated, while a kinetoplast is formed from the nucleus. The original flagellum, together with the axoneme and kinetoplast, degenerates. There is thus formed an elongated cytoplasmic body in which are embedded a number of leishmania forms. Presumably by rupture of the cytoplasm of the original parent, these escape and produce the crithidia forms again. This is a remarkable process which has not hitherto been observed. The figures, which are supposed to illustrate the process, are far from convincing, and suggest the possibility that yeasts or other structures, or even leishmania forms of the flagellate itself, may have been adherent to the surface of the organisms. Minchin and Thomson, J. D. (1915), however, mention certain structures in the cytoplasm of *Leptomonas pattoni* as evidence of a possible endogenous bud formation, a process which they also consider may occur in *T. lewisi.* They, however, never actually observed the formation of buds, and did not feel justified in describing the process.

SYNGAMY.

The possibility of a sexual process occurring in trypanosomes and their allies has attracted considerable attention. Schaudinn (1904) described syngamy in the case of *Trypanosoma noctuæ*, and Prowazek (1904, 1905) in *T. lewisi* and *Herpetomonas muscarum.* Various processes of syngamy, parthenogenesis (development of the female gamete without fertilization), and ethiogenesis (development of the male gamete without fertilization),

are included in the complicated descriptions of the latter writer. The observations, or more correctly the deductions, made by him were undoubtedly the outcome of a theoretical bias, and cannot be accepted. Influenced by these statements, numerous observers, without any evidence whatever, have described as male and female flagellates the narrow and broad forms which occur in almost every infection. The figured stages of conjugation can always be interpreted as the final stages of a division in which a narrow form, the supposed male, is separating from a broader one, the supposed female. The instance recently described by França (1920*a*) for *Phytomonas davidi* is unconvincing, as the forms figured can easily be explained as dividing individuals or the casual association of two flagellates.

As regards the pathogenic trypanosomes, Woodcock and Lapage (1915) go to the other extreme, and suggest the complete loss of syngamy in this group. It is quite possible that a sexual process takes place in association with the development of trypanosomes in their invertebrate hosts, but the most careful observations, such as those of Robertson (1913) on the development of *T. gambiense* in tsetse flies, and of Minchin and Thomson (1915) on *T. lewisi* in the flea, have failed to reveal one, though the possibility of its having escaped detection is admitted. In order to demonstrate a sexual process, something more than a casual association of two unequal forms in stained films is necessary. The formation of "latent bodies," as described by Breinl and Moore, and referred to above, was supposed by them, without any real evidence, to depend on a fusion of the kinetoplast and nucleus, and to represent a process of self-fertilization or autogamy. Roubaud (1911*c*, 1912*b*) noted that in certain insect flagellates, when they became leishmania forms in the hind-gut in preparation for encystment, the kinetoplast approached the nucleus. He concluded that actual fusion of the two sometimes took place, and that a process of autogamy was represented. It is very doubtful if any fusion occurs, as the disturbing effect of drying the parasites on films so obscures the true nuclear structure that it is impossible to be certain that any apparent fusion of two closely applied bodies is not merely artificial. Quite apart from the defects due to drying, it often happens that the kinetoplast lies over the nucleus, and it becomes impossible to distinguish the two as separate bodies in such minute organisms. Even if such a fusion as that described takes place, there is no evidence that it represents syngamy in any form.

It is clearly evident that, of the numerous statements which have been made regarding the occurrence of sexual differentiation and syngamy amongst the Trypanosomidæ, not one has any evidence to support it.

ENCYSTATION.

In those Trypanosomidæ which are confined to insect hosts, infection is spread from one to another by encysted forms passed in the fæces. In the trypanosomes, however, cysts do not occur, as infection takes place either by the inoculation of free forms through the proboscis, or by the ingestion by the vertebrate of free forms passed in the fæces of the insect. The cyst, in the case of the purely insect flagellates, is formed in the hind-gut or rectum around small leishmania forms which are produced there by the gradual shortening of the elongated forms (Fig. 150, 13–16, 24–26, 46–50). They are described as differing from the unencysted leishmania forms in that they have a much more definite and deeply stained outline. In some cases the cyst wall is depicted as quite thick, and even radially striated. It may be very difficult to distinguish the kinetoplast from the nucleus, the two structures often lying very close together or in complete apposition. The process of encystment in *Herpetomonas muscarum* was described by Prowazek (1904), and by Minchin (1908) in *H. grayi* (Fig. 173, 10–16). In the latter the cysts, which were called " Schleimcysten " by Prowazek, commence to form around the blunt posterior end of the flagellate, which is still in the elongate flagellate state. As the cyst forms, the organism becomes more and more retracted and the flagellum withdrawn, till finally it becomes a pear-shaped structure in which the flagellum is represented only by the short axoneme in the cytoplasm. The cyst then closes round the more pointed anterior end. It is at first of a gelatinous nature, and encloses a cytoplasm in which the nucleus and kinetoplast can be distinguished. The axoneme finally disappears, leaving in its place an area which stains red with Giemsa stain, and this in its turn vanishes also. The nucleus and kinetoplast become broken up into separate granules, so that their identity is difficult to make out unless the more deeply staining ones are derived from the kinetoplast. The cyst, at first pear-shaped, becomes more circular in outline, the more or less spherical condition being the final one. There seems, however, to be some doubt as to the nature of the structures called cysts in the case of *H. grayi*. Koch (1906) suggested that the flagellate was possibly the developmental form of the crocodile trypanosome, while Minchin (1908) thought it possibly represented a bird trypanosome. Kleine (1919*a*) claims that the flagellate is actually derived from the crocodile trypanosome, as tsetse flies bred in the laboratory acquire an infection when fed upon crocodiles harbouring this trypanosome; while Lloyd, Johnson, Young, and Morrison (1924) have produced evidence that it may be derived from either the crocodile, monitor, or toad. If, then, the so-called cysts are actually true cysts, and not merely leishmania

forms—which, owing to deposit round them on the film, have taken on the appearance of cysts—it has to be assumed that the flagellate of the tsetse fly not only passes from fly to crocodile, but also from fly to fly by means of cysts; or that the crocodile becomes infected by ingesting the cysts passed in the fæces of the fly. The discovery by Lloyd (1924) of a typical *Leptomonas* in the labial cavity of the proboscis and the mid-gut of *Glossina morsitans* still further complicates the question of the nature of the so-called cysts of these flagellates.

In the process of encystment of *H. muscarum*, the flagellate first retracts itself to an ovoid body around which the cyst forms. The retraction, however, may take place in three different ways, as will be described below (Fig. 171). That the cysts of insect flagellates are actually resistant bodies has been proved by the writer (1912*c*) in the case of the leptomonas of the flea, *Pulex irritans* (p. 351). It must be remarked, however, that it is exceedingly difficult in most cases to form a definite opinion as to whether a minute leishmania form, as seen in the hind-gut of an insect, is actually encysted or not, and the mere fact that in dried films stained by Romanowsky stain a red line surrounds them can hardly be regarded as evidence of the presence of a cyst wall. The thick envelopes which have been figured as cyst walls by numerous observers are probably artefacts due to the staining of granular material which has become heaped round the leishmania forms in the process of drying the films. In other cases, the structures described as cysts have probably been yeasts, or even spores of microsporidia. That a membrane actually exists seems to be proved by the resistance of these forms to drying, but it must be admitted that the detection of a cyst wall is a far more difficult matter than many have supposed. Hoare (1923), in his work on the development of the trypanosome of the sheep in the ked, draws attention to the possibility of mistaking yeasts or other artefacts for cysts, and has shown that the supposed cysts of the flagellate of the ked, which was at one time thought to be a parasite peculiar to this insect, are merely rounded forms, with deposits of stained granular material round them, or yeasts. The cysts of *H. grayi* may be capable of a similar interpretation (Fig. 173). The changes in nuclear structure which occur during the alleged encystation of this flagellate may be merely evidence of degeneration.

GENERAL FEATURES OF THE LIFE-HISTORY.

In the description of genera given above it has been explained that the flagellates belonging to the genera *Herpetomonas*, *Crithidia*, and *Leptomonas* have only an invertebrate host, while those belonging to the genera *Trypanosoma* and *Leishmania* have both vertebrate and inverte-

brate hosts. As regards the forms which occur in vertebrates, it will be found that the organisms may assume any of the forms between the trypanosome and the leishmania types. In the blood or other fluids of the body they are usually provided with flagella, and have the trypanosome structure, but occasionally, as in the case of *Trypanosoma lewisi*, free-swimming forms of the crithidia or leptomonas type occur also. If the flagellates are intracellular, they tend to be of the leishmania type, as in the case of *Leishmania donovani*, *L. tropica*, and *T. cruzi*. In the case of *T. cruzi*, however, after a number of intracellular leishmania forms have been produced, they gradually become transformed through a crithidia phase into flagellates having the trypanosome structure, while maintaining their intracellular position. Any of these various forms, whether free-swimming in the fluids of the body or in the cytoplasm of cells, can reproduce by binary fission.

In the invertebrate host, the members of the genera *Trypanosoma* and *Herpetomonas* may occur in any form between the leishmania and the trypanosome. The members of the genus *Crithidia* never pass beyond the crithidia stage, while those of the genus *Leptomonas* never pass beyond the leptomonas stage. Multiplication of all these forms by binary fission takes place as in the vertebrate. During the development in the invertebrate, the flagellates may be provided with flagella, by means of which they swim freely in the lumen of the gut, proboscis, salivary gland, or in other situations. These free forms were termed *nectomonads* by Minchin and Thomson (1915). On the other hand they may attach themselves to the lining cells by their anterior extremities, in which case the flagella are lost, but in the arrangement of the nucleus, kinetoplast, and axoneme they may have the trypanosome, crithidia, leptomonas, or leishmania structure. Such attached forms have been called *haptomonads* by Woodcock (1914). The attached forms may retain their elongate character in the anterior portions of the intestine. In the hind-gut there is a general tendency for the elongate flagellates to become much shortened, though the nuclei and kinetoplasts may retain their relative positions. The haptomonad forms occur very commonly in the case of those flagellates which are limited entirely to invertebrates, but they are also found in the invertebrate phase of development of trypanosomes. Thus, they occur in the case of *T: lewisi* in the hind-gut of fleas, *T. vivax* in the proboscis of *Glossina morsitans*, and *T. gambiense* in the salivary glands of *G. palpalis*.

The flagellates belonging to the genera *Herpetomonas*, *Crithidia*, and *Leptomonas* have but a single invertebrate host, infection being spread by means of encysted leishmania forms passed in the dejecta. When such an encysted form is eaten by a new host, the liberated leishmania form gradually grows to the adult flagellate form. During this period of

growth, multiplication by binary fission may occur. The various forms which occur before the adult flagellate stage is reached have been called *pre-flagellates* by Patton (1908*b*). When the fully-formed flagellate stage has persisted and reproduced for some time, there occurs a gradual retraction of the body towards the leishmania form in preparation for encystment. The forms leading to encystment have been called *post-flagellates.* These are usually attached to the surface of the cells lining the hind-gut, and are thus haptomonad forms. In the case of the development of trypanosomes in the invertebrate, the final infective forms which pass back to the vertebrate have the trypanosome structure, and have been developed from attached or haptomonad forms of the crithidia type. These infective forms have been termed *metacyclic trypanosomes* by Brumpt (1913). In the case of some trypanosomes (*T. lewisi*, *T. cruzi*, *T. melophagium*), they are produced in the hind-gut of the invertebrate and escape in the fæces, which are ingested by the vertebrate (development in the posterior station); while in others (*T. gambiense*, *T. vivax*, *T. granulosum*) they develop in the anterior part of the alimentary tract, in the salivary glands (tsetse flies), or proboscis sheath (leeches), and enter the vertebrate during the biting act (development in the anterior station).

CLASSIFICATION.

The classification of the members of this family is a difficult one on account of the many gaps in knowledge and the contradictory statements made by different observers. The flagellates, which are limited entirely to invertebrate hosts, are handed on from one to the other by encysted forms in the fæces. Those which have both a vertebrate and invertebrate host, as far as is known, always pass from the latter to the former in the unencysted condition. In the case of flagellates of the genus *Trypanosoma*, the infective forms are of the trypanosome type (metacyclic trypanosomes). The exact form which is infective in the case of members of the genus *Leishmania* is not known, but it may be assumed that encysted forms are, at any rate, unnecessary. Assuming this to be the case, it is possible to divide the members of the family into two groups—those limited entirely to invertebrates, in which infection is contaminative through one insect ingesting cysts passed by another (*Leptomonas*, *Crithidia*, *Herpetomonas*), and those occurring in both vertebrate and invertebrate hosts, in which infection is passed from the invertebrate to the vertebrate by the former inoculating unencysted flagellates during the act of feeding or passing unencysted flagellates in its fæces, which either contaminate the puncture wound or are eaten by the vertebrate (*Leishmania*, *Trypanosoma*). The flagellates with two hosts can be divided into two groups according as the highest stage of development is the leptomonas

form (*Leishmania*) or the trypanosome form (*Trypanosoma*). The latter, again, can be subdivided into those forms, such as *T. lewisi*, which are carried by fleas; *T. cruzi*, conveyed by reduviid bugs; *T. melophagium*, transmitted by the sheep ked; and possibly *T. theileri* and its allies, the invertebrate hosts of which are probably tabanid flies and the trypanosomes of land reptiles, including crocodiles, in which development in the invertebrate leads to the formation of metacyclic trypanosomes in the hind-gut, and passage of these in the fæces (development in the posterior station); or into those like *T. gambiense*, *T. vivax*, and *T. congolense*, and the trypanosomes of some cold-blooded vertebrates, the development of which in the invertebrate results in the formation of metacyclic trypanosomes in the region of the proboscis and their inoculation during the biting act (development in the anterior station). The trypanosomes which develop in the anterior station, a term first proposed by Duke (1913), can further be grouped into those developing in biting flies (the pathogenic trypanosomes of mammals) and those which develop in leeches (the trypanosomes of aquatic reptiles, amphibia, and fish). The trypanosomes of birds are difficult to place, for some have claimed that development takes place in the mosquito, and that they are inoculated at the time the mosquito bites. On the other hand, it seems very probable that the true host of the bird trypanosomes will be found amongst the ectoparasites which infest the young in the nest, and it is possible that infection may be contaminative, as in *T. lewisi*. For this reason, in the scheme of classification given below, the trypanosomes of birds have been placed in both groups with a note of interrogation. Similarly, the trypanosomes of land reptiles, including crocodiles, are placed in both groups, for it is not definitely known whether the development is in the anterior or posterior station, though the latter is probable in the case of the trypanosomes of the crocodile and the monitor which develop in tsetse flies.

The trypanosomes which develop in biting flies in the anterior station include the pathogenic forms of tropical Africa, which are conveyed by species of *Glossina* (tsetse flies), and possibly the pathogenic forms of the *T. evansi* type, including similar forms in many parts of the world, which are conveyed by Tabanidæ and their allies. It has not been actually demonstrated that *T. evansi* develops in the anterior station in tabanid flies, though its similarity to *T. brucei* renders this not improbable. A development in the posterior station is, however, possible.

As regards those which develop in tsetse flies, it will be shown below that three types of development occur, as pointed out by Duke (1913) and Bruce (1914). In one type the process commences in the stomach, but the infection spreads forwards to the proboscis and ultimately to the salivary glands, in which infective metacyclic trypanosomes are produced.

In the second the stomach phase occurs, and is followed by invasion of the proboscis, but not the salivary glands. In the third the whole development occurs in the proboscis, there being no stomach phase. As far as present knowledge goes, the trypanosomes of cold-blooded vertebrates, with the exception of those of land reptiles and crocodiles, develop in leeches. There is a stomach phase leading to invasion of the proboscis and proboscis sheath, from which trypanosomes escape into the wound as the leech feeds. Finally, there is *T. equiperdum*, in which an invertebrate host is at all events unnecessary, the infection being handed directly from vertebrate to vertebrate. This trypanosome is undoubtedly allied to those transmitted by biting flies, and evidence has been produced that infection can be sometimes spread by the agency of these insects. The flagellates of the leptomonas type parasitic in euphorbias, which have both an insect and plant host, have been separated under the generic name *Phytomonas.*

The classification outlined above and arranged in tabular form below has the advantage of convenience, if nothing more. It, however, recognizes what is definitely known about these flagellates, and probably indicates their phylogenetic history. The leishmania are probably derived from insect leptomonas, and the trypanosomes from a crithidia or herpetomonas. Those which have a development in the anterior station may have arisen by direct inoculation into the blood, while those with a posterior station may have infected the vertebrate in the first instance by way of the alimentary canal. It is known that certain lizards harbour leptomonas in the intestine. It is very probable that this infection is acquired from the insects on which the lizards feed. Similar flagellates occur in the blood of lizards, and the natural inference is that they have invaded the blood-stream from the intestine. If the insects, which were responsible for the intestinal infection, were accustomed to suck the blood of lizards, it would be possible for them to become infected from the blood, in which case they might or might not lose the power of becoming infected by ingesting the fæces of infected insects of their own kind.

TABULAR CLASSIFICATION OF THE FLAGELLATES OF THE FAMILY TRYPANOSOMIDÆ.

A. Flagellates with only an invertebrate host. Infection is contaminative by means of cysts.

(a) *Leptomonas.*
(b) *Crithidia.*
(c) *Herpetomonas.*

B. Flagellates with both a vertebrate and an invertebrate host. Infection is contaminative or inoculative. No cysts occur.

- (*a*) Flagellates in which the highest development is the leptomonas type. Infection of the vertebrate is either inoculative or contaminative. (*Leishmania.*)
- (*b*) Flagellates in which the highest development is the trypanosome type. (*Trypanosoma.*)
  1. Trypanosomes which in the invertebrate develop in the posterior station. Infection of the vertebrate is contaminative.
     - (*a*) *T. lewisi* and other similar forms in small mammals.
     - (*b*) *T. cruzi.*
     - (*c*) Non-pathogenic trypanosomes transmitted by keds, species of *Tabanus*, or other biting flies. *T. melophagium*, *T. theileri*, *T. ingens.*
     - (*d*) Trypanosomes of birds (?).
     - (*e*) Trypanosomes of land reptiles (?).
  2. Trypanosomes which in the invertebrate develop in the anterior station. Infection of the vertebrate is inoculative.
     - (*a*) Trypanosomes transmitted by blood - sucking arthropoda.
       - (1) Pathogenic trypanosomes transmitted by species of *Glossina.*
         - (*a*) Development in the stomach, proboscis, and salivary glands. *T. gambiense*, *T. brucei* (*T. rhodesiense*).
         - (*b*) Development in the stomach and proboscis. *T. congolense*, *T. simiæ.*
         - (*c*) Development in the proboscis only. *T. vivax*, *T. uniforme*, *T. capræ.*
       - (2) Pathogenic trypanosomes transmitted by species of *Tabanus* or allied flies, and possibly by ticks. *T. evansi*, *T. equinum.*
       - (3) Trypanosomes of birds (?).
       - (4) Trypanosomes of land reptiles (?).
     - (*b*) Trypanosomes transmitted by leeches.
       - (1) Trypanosomes of aquatic reptiles.
       - (2) Trypanosomes of amphibia.
       - (3) Trypanosomes of fish.

C. Pathogenic trypanosomes usually passing directly from vertebrate to vertebrate (*T. equiperdum*). (As these have undoubtedly become secondarily adapted to this mode of transmission, it might be more logical to group them with the pathogenic forms transmitted by biting flies.)

D. Flagellates with both an invertebrate and a plant host (*Phytomonas*). *P. davidi* and similar forms.

SYSTEMATIC DESCRIPTION OF GENERA AND SPECIES.

Genus: Leptomonas Kent, 1880.

The genus *Leptomonas*, as defined above, includes flagellates which in their life-cycles exhibit both leishmania and leptomonas forms, and which are confined to invertebrate hosts.

Bütschli (1878) described a flagellate which he found in the gut of a nematode (*Trilobus gracilis*), and Kent (1880) named it *Leptomonas bütschlii* as the type of the genus. Unfortunately, this flagellate has not been studied in the light of present knowledge, so that it is still uncertain if it conforms with the definition of the genus *Leptomonas* given above.

A very large number of species have been discovered in invertebrate hosts, mostly arthropods, and of these anything like a complete life-history is known only in a few instances. The form seen by Bütschli in the nematode *T. gracilis* has already been mentioned. Chatton (1924) records one seen by him in a marine nematode. Amongst the Mollusca, Porter (1914) described *L. patellæ* from the limpet *Patella vulgaris*, and Mello (1921) *L. pachylabræ* from another mollusc, *Pachylabra mœsta*.

Leptomonas ctenocephali (Fantham, 1912).—Though Patton (1908*c*) had seen a leptomonas in the Indian flea, *Ctenocephalus felis*, and its larvæ, the flagellate of the dog flea, *C. canis*, was first seen by Basile (1910*a*), who mistook it for developmental forms of *Leishmania donovani*. The same error was made by Basile and Visentini (1911), Sangiorgi (1911), Marzocchi (1911), and Alvarez and da Silva (1911). The rounded leishmania stages of the parasite were seen by Swellengrebel and Strickland (1910). Nöller (1912*d*, 1914) discovered the flagellate in dog fleas and their larvæ in Germany, and concluded that it was a specific parasite distinct from *L. donovani*. He studied the infection in fleas, and noted that it was usually confined to the hind-gut, which was often completely lined with attached forms. Fantham (1912) proposed the name *Herpetomonas ctenocephali* for the flagellate, and Brumpt (1913) the name *H. pseudoleishmania*. The writer (1913*a*) observed the flagellate in dog fleas in England, and later (1914*a*) in Malta, while da Silva (1913) studied it in connection with attempts to transmit kala-azar in Portugal. Laveran and Franchini (1919), Chatton (1919), Tyzzer and Walker (1919), Shortt (1923), and Drbohlav (1925) studied cultures of the flagellate, and noted that they differed from those of *L. donovani*.

Though the flagellate of the dog flea is named *Leptomonas ctenocephali*, it must be recognized that morphologically indistinguishable forms have been previously described and named from other fleas, and if these should be proved to be identical with that in the dog flea, the name given to the

form in the dog flea will become a synonym. The first-named form is one which Mackinnon (1909) described in *Ctenophthalmus agyrtes*, and which she named *Herpetomonas ctenophthalmi*. Swingle (1911) gave the name *H. pattoni* to one which he found in species of *Ceratophyllus* and *Pulex*, while Chatton and Delanoë (1912*a*) identified as this species a form in the larvæ and adults of *C. fasciatus*. Brumpt (1913) gave the name *H. debreuli* to a flagellate of *C. sciurorum*, and Laveran and Franchini (1915) the name *H. ctenopsyllæ* to one in *Ctenopsylla musculi*. Patton and Rao (1921) gave the name *H. pulicis* to the form in the human flea, *P. irritans*, but it is a synonym of *Crithidia pulicis*. This form, again, was first seen by Basile (1911*a*), who regarded it as *L. donovani*. Similar

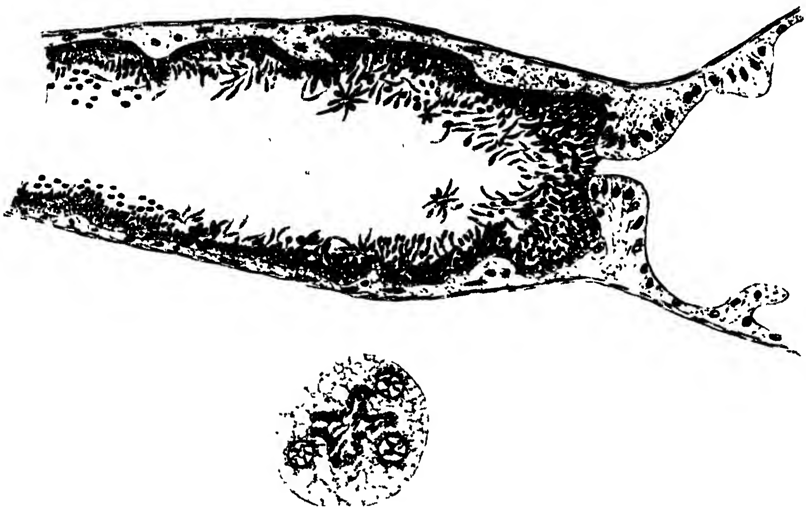


FIG. 163.—LONGITUDINAL SECTION OF THE INTESTINE AND TRANSVERSE SECTION OF A MALPIGHIAN TUBE OF THE DOG FLEA, SHOWING *Leptomonas ctenocephali* LINING THE HIND-GUT AND THE MALPIGHIAN TUBE (× *ca.* 170). (ORIGINAL.)

flagellates have been seen in other fleas, but as far as is known they correspond very closely with *L. ctenocephali*, and it is not improbable that they may be identical with it. Cross-infection experiments with bred fleas will have to be undertaken before this is finally settled. All these forms belong to the genus *Leptomonas*, as here defined.

In the dog flea the infection is limited to the intestinal tract and the Malpighian tubes which open into it just behind the stomach (Fig. 163). Most usually, flagellates do not occur in the stomach, but when the infection is exceptionally heavy, it may extend forwards to this portion of the intestine. As a rule, the infection stops abruptly at the pyloric opening, where a large cluster of free and attached organisms often occurs. The condition in which the flagellates are found in the gut depends to some

extent on the amount of blood present. The flagellates have a marked tendency to attach themselves to the lining epithelium, which may be completely covered with a mosaic of flagellates, mostly of a stumpy type. It is by the flagellar end that attachment is made, and the flagellum becomes much reduced in length till it is represented only by the axoneme; the anterior end of the organism then lies in contact with the epithelial cell. The majority of flagellates are attached to the epithelium, and this is probably a result of the behaviour of the gut when the flea feeds. During this act, by means of transmitted light, the gut can be seen to be in a state of violent peristalsis, the waves passing first in one direction and then the other. The result is that the first droplet of liquid ejected from the rectum by the flea contains pure unaltered blood, and if the flea has been feeding on a rat infected, for instance, with *Trypanosoma lewisi*, the living trypanosomes may be found in the first droplet passed. It is clear that if all the leptomonas were free in the gut cavity, the majority would be voided with the dejecta. Only those forms which are free or have become detached escape in the ejected blood, and in this all the various stages of the flagellate which occur in the gut can be found. When there is little nourishment in the hind-gut, practically all the flagellates are in the attached condition, but after a meal of blood, many active flagellates can be seen in the gut contents, the long flagellate forms being developed from the shorter non-flagellate attached ones. The infection may spread into the Malpighian tubes, where the same series of free and attached forms are to be found. Towards the posterior end of the intestine the attached flagellates, and also those free in the cavity, become smaller, till finally little ovoid leishmania forms are produced. These, together with all the larger forms up to the longest flagellates, are found in large numbers in the fæces of the flea, which consist of droplets of digested, semi-digested, or pure blood. The flea has such a voracious appetite that it will continue to feed for a long time, filling its stomach again and again with fresh blood, while it repeatedly voids what is apparently pure blood from its rectum. The general rule is that the largest flagellates are found in the fore part of the hind-gut, either free or attached, and the smallest forms in the rectum, but this rule is not absolutely constant. Sometimes the whole gut is lined with short stumpy forms with very few long forms, at others there is a larger number of long forms. In attachment there is a tendency for groups of flagellates to be arranged as a disc, with the flagella directed towards the centre or in a hemispherical mass, the so-called rosette, which has its base on the epithelium, the flagella of the individual flagellates being directed centrally. Such groups increase in number till the whole gut is covered. In these groups all individuals may be long or short forms, or a single group may show every transition from the largest

flagellate forms to the smallest rounded ones which have no free flagellum. The clusters or rosettes of attached forms increase in size by multiplication of the individual flagellates, which are able to divide longitudinally in whatever form they occur.

The small leishmania forms which arise in the hind-gut appear to develop a cyst wall. The absolute proof of the existence of a cyst in such minute forms is, of course, difficult to obtain, though the writer (1914*a*) has shown that these supposed encysted forms are protected in some way against desiccation. The fæces of an infected flea, which were passed while feeding, were received on to a sterile cover-glass held a short distance behind it. The droplet was spread into a thin film with a sterile needle and allowed to dry. The cover-glass was then placed in a dry sterile test-tube for twenty-four hours, after which it was transferred to N.N.N. medium, in which a culture of the flagellates was obtained. A similar experiment was made by the writer (1912*c*) with the flagellate of the human flea, *Pulex irritans*. This is sufficient evidence to show that in the fæces there occur forms which can withstand complete drying, and in all probability these are the small apparently encysted leishmania bodies. It is assumed that the flagellates and unencysted forms must be killed in the process of drying.

It is well known that the larvæ of these fleas feed largely on the fæces of the adults, and, as demonstrated by Nöller (1914), they take up the small cysts, for the same flagellates can be found in their intestine. Here, also, both elongated and shorter forms occur, but the writer has never seen the gut covered with attached flagellates, as in the adult. Drbohlav (1925) has shown that the flagellate infection of the larvæ survives in the pupæ, and appears as an intestinal infection in the newly-emerged adults.

It will be seen that the infection is a simple one, which passes from one insect to another by means of encysted forms voided in the fæces.

The various types of organism from the flea's intestine are shown in Fig. 164. The longest forms have a body 18 microns in length. There is a distinct tendency to curvature like the blade of a curved sword. Very narrow forms occur, as also much broader ones, and between the long flagellate forms and the minute leishmania ones every stage can be traced. The small encysted bodies which are finally produced are barely 3 microns in diameter. Reproduction takes place by binary fission, and this is not confined to any particular stage, flagellates of all sizes and shapes taking part in the process. No stage of intracellular reproduction corresponding with that of *Trypanosoma lewisi* in the epithelial cells of the flea's gut has been seen in this flagellate or in any other leptomonas.

There is no evidence that *L. ctenocephali* has any vertebrate host, in spite of the claims, which appear somewhat dubious, of Laveran and

Franchini (1913*a*) that they were able to infect mice. These experiments will be considered more fully below. The presence of *L. ctenocephali* in fleas led Basile and others to the view that *Leishmania donovani* undergoes a development in the flea, the natural flea flagellate being mistaken for developmental forms of the parasite of kala-azar.

As will be seen in the lists of hosts, leptomonas have been found in a number of fleas, and some of these have been given specific names without

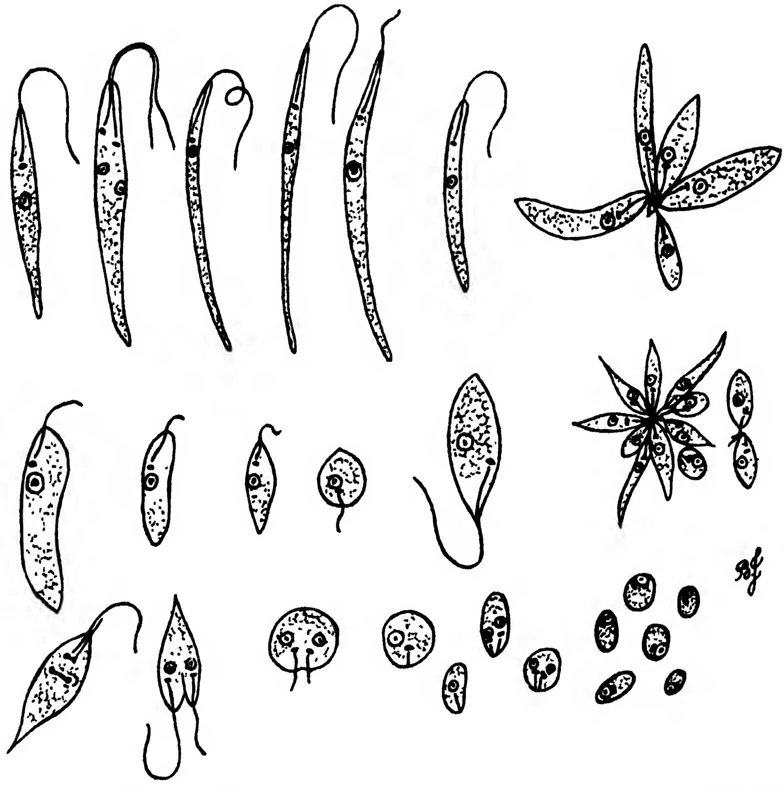


FIG. 164.—*Leptomonas ctenocephali* FROM INTESTINES OF DOG FLEA (*Ctenocephalus canis*) (×2,000). (ORIGINAL.)

At bottom right-hand corner are the presumably encysted forms which occur in fæces.

there being any real justification for this procedure. The form in the human flea, *Pulex irritans*, was studied by the writer (1912*c*), and was named *L. pulicis* by Patton and Rao (1921). They found fleas naturally infected, and also succeeded in infecting fleas experimentally by feeding them on cultures of the flagellate. Larvæ kept with infected fleas became themselves infected. They ingest the rounded forms passed in the fæces of the adult, and acquire an infection of the stomach in which the flagellates live and

multiply. The flagellates survive the pupal stage, and in the adult flea appear in the hind-gut and Malpighian tubes. The various forms found in the adult fleas are described as pre-flagellates, flagellates, and post-flagellates. Certain round forms, called pre-flagellates, are described from the Malpighian tubes of the flea, and to account for their presence the improbable assumption is made that they have been carried there by adhering to flagellate forms which have migrated from the gut. It would be expected that the pre-flagellates would only exist in the larvæ, as it is admitted that the flagellates develop in the stomach of the larvæ. The pre-flagellates, it will be remembered, are the rounded forms which result from the ingested encysted stages, and which develop into the full-grown flagellates. If they occur in the adult flea, one must suppose that they have not completed their development in the larvæ which ingest them, as some of them are admitted to do, and that they have passed through the pupal stage of the flea. The occurrence of these forms in the adult flea rather suggests that the forms pre-flagellate, flagellate, and post-flagellate, which Patton describes in this and other insect flagellates, do not follow one another in succession so regularly as he supposes. It seems more probable that the flagellates may become rounded leishmania forms, which may again develop into flagellates in the same host, without necessarily passing on to the encysted stage, to be voided in the fæces.

As already remarked, cultures of *L. ctenocephali* and the flagellates of other fleas can readily be obtained on N.N.N. medium by receiving the voided droplets of liquid fæces of fleas on sterile cover-glasses and transferring them to the culture fluid. The writer (1914*a*) obtained such cultures from dog fleas in Malta. Laveran and Franchini (1919), Chatton (1919), Tyzzer and Walker (1919), and Shortt (1923) have obtained cultures by washing the fleas in sterilizing fluids and dissecting them under aseptic conditions. These cultures grow readily, can be maintained by subculture for any length of time, and show all the forms which occur in the insect gut. There is never any tendency towards the formation of crithidia or trypanosome forms. Growth is very rapid, much more so than in the case of the allied pathogenic leishmania. The cultures remain alive for long periods, and enormous numbers of flagellates are produced. In one instance in the writer's experience, active flagellates were still present six months after the tube of N.N.N. medium had been inoculated, and a subculture was obtained from it two months later, when active flagellates had disappeared, though leishmania forms were still present. In old cultures, many abnormal and evidently degenerating forms occur.

Tyzzer and Walker (1919) made a careful comparative study of cultures of *Leishmania donovani* (Mediterranean strain) and *Leptomonas ctenocephali*. The flea flagellate grew more rapidly at 21° C. than *L. donovani*,

while it still multiplied at 10° C., whereas *L. donovani* did not. *L. ctenocephali* showed a greater tendency to grow in clumps with the flagella internally directed, and it was generally more active than *L. donovani*. The fully-grown flagellates of *L. donovani* varied in length from 9 to 12·5 microns, with flagella 7·5 to 15·3 microns long. The corresponding stages of *L. ctenocephali* measured 11 to 16·8 microns, with flagella 7·3 to 21 microns in length. In the case of *L. ctenocephali*, the long forms frequently had the aflagellar end extremely attenuated or ribbon-like, while spiral twisting of this part of the body was common. In *L. donovani* the nucleus was centrally situated, with the kinetoplast near the anterior end

FIG. 165.—*Leptomonas pulicis* OF THE HUMAN FLEA (*Pulex irritans*). (ORIGINAL). A cluster of flagellates from a culture in N.N.N. medium (× 2,000).

of the body, while in *L. ctenocephali* the nucleus was definitely in front of the middle of the body and the kinetoplast was near it. Chatton (1919) had already drawn attention to the long, acicular forms which occurred in cultures of *L. ctenocephali*, and which were absent from cultures of *L. donovani*. Drbohlav (1925) has shown that dog fleas may be infected with *L. ctenocephali* by injecting them *per rectum* with cultures, or by allowing them to feed on cultures through a membrane. The cultures of *L. pulicis* of the human flea studied by the writer (1912*c*) are very similar (Fig. 165).

Laveran and Franchini (1919, 1920) claim to have produced a generalized infection of mice and guinea-pigs by inoculating them with

cultures of *L. ctenocephali*. They state that a local infection occurred in one guinea-pig inoculated in the testis. Nöller (1912*d*) failed to infect a dog with *L. ctenocephali*, and Chatton (1919) was equally unsuccessful with mice. Shortt (1923*a*) attempted to infect dogs, monkeys, cats, mice, pigeons, and frogs. The animals were examined by the smear and culture method, but no evidence of infection was obtained. Yamasaki (1924) also failed to infect mice and dogs, and noted that the flagellate differed morphologically from *Leishmania donovani*. Drbohlav (1925) has failed completely to produce any infection in a series of about 150 animals, including one monkey, dogs, guinea-pigs, rats, and mice. In the light of these failures the claims of Laveran and Franchini that practically every animal inoculated acquired an infection are difficult to explain.

Laveran and Franchini (1920*a*) also claim to have infected Euphorbia plants (*E. sauliana* and *E. pilosa*) by inoculating them with cultures of *L. ctenocephali*. The flagellates were said to be present in the plants for at least thirty-five days. Shortt (1923) introduced cultures of this flagellate into small pockets in *E. royleana* in India, where they survived for six days. Some of the flagellates became elongated, and showed the peculiar twisting of the posterior end of the body so characteristic of the natural Euphorbia flagellate.

By feeding bed bugs on cultures of the leptomonas of *Pulex irritans* and cultivating from the intestine, Patton, La Frenais, and Rao (1921) have shown that the flagellates can survive in the bug at least thirty-seven days. Shortt (1923) has also shown that active multiplication of *L. ctenocephali* takes place in the stomach of bed bugs fed on cultures. Up to forty-eight hours there may be a very heavy infection of the stomach, after which it subsides, till in eight days very few flagellates occur. Flagellates may, however, still be present in the hind-gut. Multiplication of the flagellate will also take place for a few days in bugs which have died after feeding. The effect of giving the bugs feeds of blood after ingestion of the culture has not been tried.

Genus: Crithidia Leger, 1902.

This genus was first created by Leger, L. (1902*a*), for a flagellate (*Crithidia fasciculata*) which he had found in *Anopheles maculipennis*. The name was based on the short, stumpy, leishmania forms which Léger considered characteristic of this genus. As, however, these forms occur in flagellates of the genera *Leptomonas* and *Herpetomonas*, this character cannot be considered of generic value. Léger's genus *Crithidia* was emended by Patton (1908*a*) in accordance with the definition given above. The flagellates of this genus are purely parasites of invertebrates, and in their most highly developed form are elongated organisms with a

rounded posterior and tapering anterior end. The kinetoplast lies close to, but still in front of, the nucleus, while the axoneme, before entering

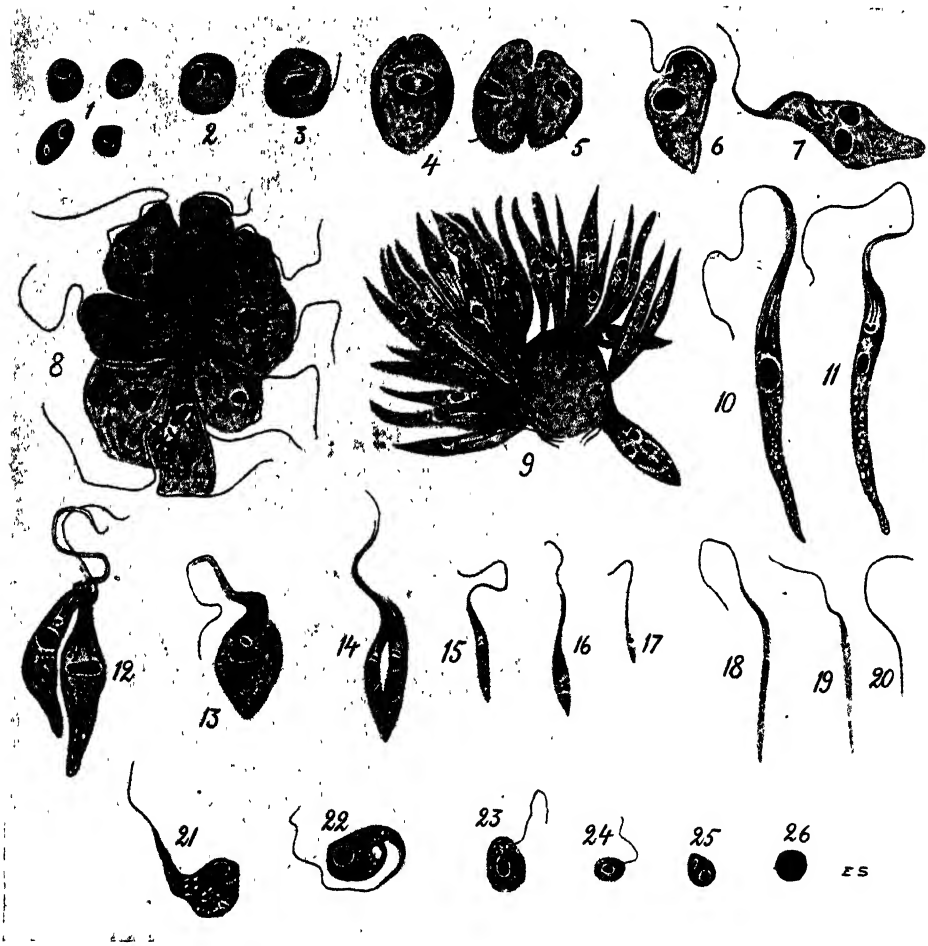


FIG. 166.—*Crithidia gerridis* FROM THE WATER BUGS, *Gerris fossarum* AND *Microvelia* sp. (No. 9 × 460; OTHERS × 800). (AFTER PATTON, 1908.)

1. Pre-flagellate forms from mid-gut of nymph.
2. Early stage of development of axoneme.
3. Development of flagellum and commencing division.
4 and 5. Dividing forms with developing flagella.
6 and 7. Forms evolving towards the crithidia type.
8. Cluster of rounded forms with developing flagella.
9. Cluster of crithidia forms adherent to a particle by their flagellar ends.
10-12. Elongate crithidia form.
13. Club-shaped crithidia forms.
14. Dividing crithidia forms.
15-20. Short and narrow crithidia forms.
21-26. Stages in development of post-flagellate forms which escape in fæces and lead to infection of young nymphs.

the flagellum, passes along an undulating membrane to the drawn-out tapering anterior end of the body. These long forms become shorter and finally converted into round leishmania forms, which appear to encyst and escape in the fæces of the invertebrate. As in the case of leptomonas, the cysts lead to infection of a new host.

Crithidia gerridis Patton, 1908.—This flagellate is an intestinal parasite of the water bug, *Gerris fossarum*, where it was first seen by Patton. It is also found in a species of *Microvelia*, and another water bug related to *Perittopus* (Fig. 166). It was chiefly studied by Patton in *Microvelia.* The alimentary canal of the *Microvelia* consists of a narrow œsophagus opening into a sacculated crop. The latter opens into the short, dilated mid-gut, which nearly always contains a greenish-yellow fluid. The mid-gut is followed by the small intestine, at the anterior end of which open four long, narrow Malpighian tubes. The small intestine is followed by the dilated colon continuous with the short, straight rectum. The eggs hatch into nymphs, which by five moults attain the adult condition. In the crop of the nymphs are found the encysted forms which have been ingested with water (Fig. 166, 1). Very shortly after their ingestion, these round forms produce flagella and begin to multiply. The smallest round forms are 4 to 6 microns in length by 3 to 4 microns in breadth. At first, these round forms possess only nucleus and kinetoplast. Very soon, from the latter the axoneme is formed, but when it reaches the surface of the body, instead of immediately entering the flagellum, it passes along the edge of a narrow undulating membrane, the rudiment of the structure which is seen fully developed in the adult crithidia forms (Fig. 166, 2–5). These forms, having increased in size, now measure 6 to 10 microns by 4 to 8 microns. Multiplication takes place at this stage by binary fission. Patton states that the flagellum actually divides longitudinally, but this is certainly incorrect. By active division rosettes of rounded flagellates are produced, with the flagella directed outwards (Fig. 166, 8). These rosettes are attached in masses to the lining epithelium. They gradually break up, and the individual flagellates swim away. The pole opposite that to which the flagellum is attached elongates, while the flagellated pole becomes drawn out with the flagellum. In this manner the typical crithidia forms arise. They vary from 15 to 45 microns in length and 2 to 4 microns in breadth (Fig. 166, 10–12). The anterior end of the body is drawn out to a fine point where the axoneme enters the flagellum. There is an undulating membrane on the part of the body anterior to the nucleus, and the axoneme passes along its margin. The posterior end of the body is rounded. The nucleus is spherical and situated at the centre of the parasite, while the kinetoplast is 1 to 1·5 microns in front of it. These long forms are often agglomerated together

by their flagellar ends, or attached to cells or débris (Fig. 166, 9). Multiplication of these forms again takes place.

Flagellates of all sizes and shapes are found not only in the crop, but also in the other parts of the intestinal tract. In the rectum there is a gradual production of short forms, by a process the reverse of that which occurred in the crops of the nymphs, by the drawing in of the anterior and posterior ends (Fig. 166, 21–26). Oval or round forms measuring 4 to 6 microns by 3 to 4 microns are thus produced. The flagellum is lost or absorbed, the axoneme alone remaining. These forms become enclosed in cysts of various sizes. Not only are these cysts voided with the fæces of the bug, but any other forms which may be present in the rectum also escape, so that it is possible the nymphs may become infected, not only by ingestion of the cysts, but of the unencysted forms also. Patton has noted that the bugs have cannibalistic habits, and often kill and feed on one another, so that infection may take place in this manner. The flagellates were never found in any other organ than the intestine, and there was no evidence that infection of offspring through the eggs could take place.

The life-history of *Crithidia gerridis* is very similar to that of *Leptomonas ctenocephali*, there being direct infection from host to host by means of cysts voided in the fæces. The difference is that the flagellates develop further towards the trypanosome type. Becker (1923, 1923*b*), in a study of *C. gerridis* in *Gerris remiges* in North America, actually noted that trypanosome forms occasionally appear. He has also seen the flagellate in *Microvelia americana*, *G. marginatus*, and *G. rufoscutellatus*.

Fantham and Porter (1916) stated that they had infected vertebrates by inoculating them with *C. gerridis*. Becker (1923*a*) has failed entirely to confirm these observations.

A cycle of development similar to that of *C. gerridis* has been described by Patton (1909) for *C. tabani* of *Tabanus hilarius* and *Tabanus* sp., and by Porter (1911) for a parasite of the human flea, *Pulex irritans*. The latter flagellate was given the name *C. pulicis*, which had previously been used by Balfour (1909*a*) for a similar, though not necessarily identical, form discovered by him (1906*a*) in the flea, *Læmopsylla cleopatræ*, in the Sudan. From the observations of Nöller (1916), it seems probable that the *Crithidia* of tabanid flies are really developmental stages of *Trypanosoma theileri* (see p. 501).

Crithidia hyalommæ O'Farrell, 1913.—This flagellate is worthy of special consideration, not only because it infects the body cavity fluid of its host, *Hyalomma ægyptium*, but also because actual infection of the ova is described as leading to infection of the hatched offspring (Fig. 167).

The parasite was found by O'Farrell in ticks living on cattle in the Anglo-Egyptian Sudan. In the first place, it might be suggested that

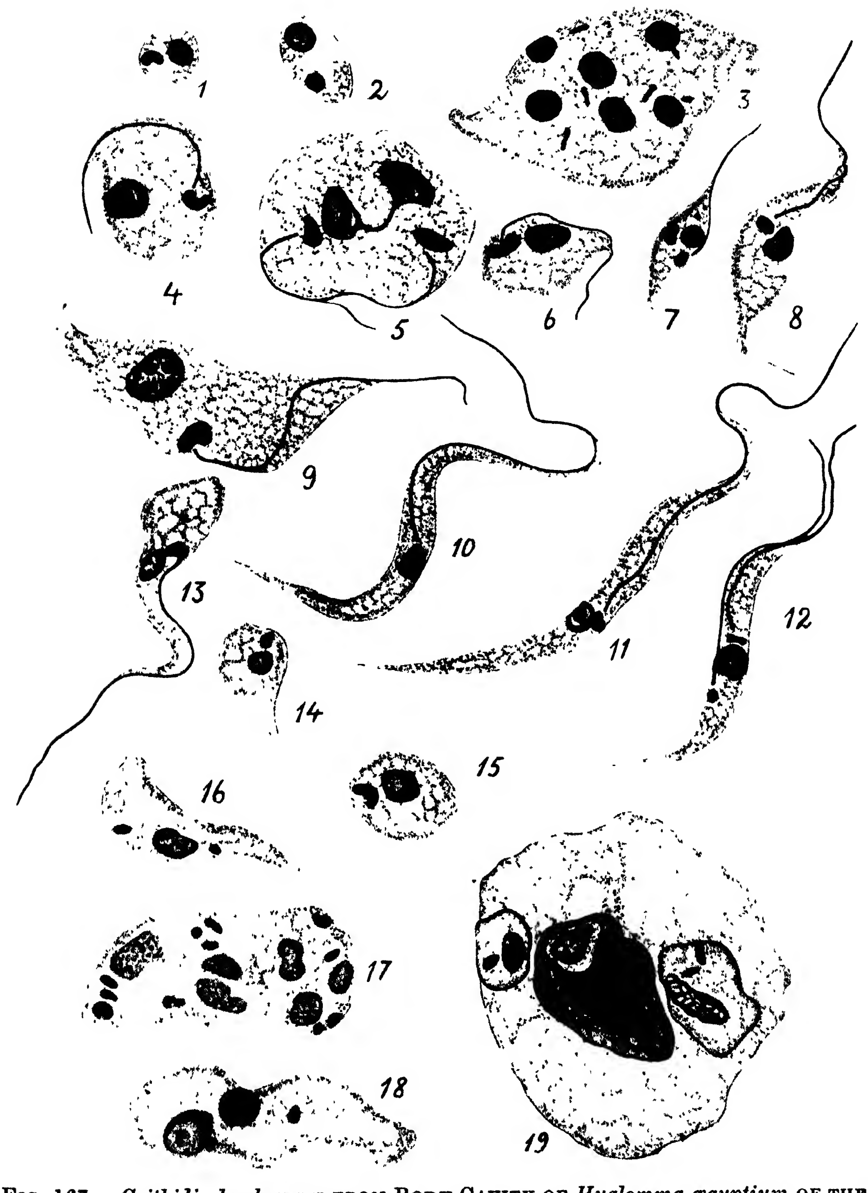


FIG. 167.—*Crithidia hyalommæ* FROM BODY CAVITY OF *Hyalomma ægyptium* OF THE SUDAN (× 2,000). (AFTER O'FARRELL, 1913.)

1-2. Leishmania forms in hæmocœle fluid (described as pre-flagellate forms).
3. Plasmodial phase from hæmocœle fluid.
4-10. Development of flagella and transformat'on into crithidia forms.
11-12. Fully-formed crithidia.
13-15. Retrogression form in hæmocœle fluid (described as post-flagellate form).
16. Ovarian form (described as post-flagellate stage).
17. Plasmodial form in ovary. 18. Form in salivary gland.
19. Stages in ovarian cell.

the flagellates represented developmental forms of a cattle trypanosome, but this was considered to be negatived from the fact that only a few ticks from any single animal were found infected. No other ticks on the animals than this particular species showed infection. The cattle, moreover, were invariably healthy. A remarkable feature of the infection is that it is not an intestinal one, but is confined to the body cavity or hæmocœle. At the height of an infection, which occurs just before the tick oviposits, the smallest drop of fluid obtained by cutting off one of the legs is found to be swarming with flagellates. In the early stages of an infection, only round leishmania forms occur, but these gradually develop into the adult crithidia forms. Multiplication takes place in the usual way, all stages of the flagellate participating in this. After oviposition, and just before the death of the tick, round leishmania forms (post-flagellate forms) may appear in the fluid. The intestinal diverticula and Malpighian tubes were not found to harbour the parasite, though as non-flagellate forms they were sometimes found in the salivary glands, but this was exceptional. Infection of the ovaries is described as taking place by the flagellates piercing the walls of the oviducts, and then entering the ova. Some of the flagellates remain in the cells of the oviducts, where they become transformed into leishmania forms. Those that enter the eggs likewise become of the leishmania type, and here they may be seen in process of division. It is unfortunate that, in his account of this developmental process, O'Farrell does not make any reference to the examination of the newly-hatched young. Examination of the ovaries by the section rather than the smear method would have given more trustworthy results as regards the supposed invasion of the eggs. As the author says, the hæmocœle fluid became " a felted mass of crithidial bodies and waving flagella," and it must be difficult in such a case to exclude the contamination of the interior of an egg with hæmocœle fluid when smears are made.

Several other instances of infection of ova by flagellates are on record, but in all cases the smear method was used, though Porter (1909*b*) claims to have actually observed penetration of the egg of *Nepa cinerea* by the living flagellate forms of *Leptomonas jaculum.* Flu (1908), Swingle (1909), and Porter (1910) have described invasion of the ova of *Melophagus ovinus*, the sheep ked, by the flagellates of these insects. Porter (1909*b*, 1909*c*), though claiming to have observed *L. jaculum* and *C. gerridis* within the eggs of their hosts, considers that they degenerate without infecting the egg. This condition is supposed to lead up to that in *M. ovinus*, where invasion of the ova is said to be followed by multiplication, so that hereditary infection occurs. The statements regarding *M. ovinus* can hardly be accepted in view of the fact that it is now known that the

flagellate, which was supposed to be peculiar to the ked, is the developmental form of the sheep trypanosome. Hoare (1921*a*), working in the writer's laboratory, could find no evidence of invasion of the eggs of either *M. ovinus* or *N. cinerea*, but noted the accumulation around the ova of spermatozoa, which produced an appearance of discarded flagella. It is possible that these were mistaken for the flagella of flagellates.

Prowazek (1912*b*) described infection of the egg of *Sarcophaga* by *L. sarcophagæ*, but it is not clear that intestinal contamination was avoided. It is evident the question of transmission of flagellates through the ova requires to be studied by the more accurate method of sectioning the ovaries and eggs. In the case of such a host as *Hyalomma ægyptium*, which, apparently, only sucks blood, it would appear that ovarian infection would be the only method of transference from host to host if the possibility of a cattle trypanosome is excluded. In this connection it must not be forgotten that the apparently harmless *Trypanosoma theileri* is often present in cattle in such small numbers that it can only be demonstrated by culture methods. This source of the infection in the tick has not been considered, nor is mention made of any infection in the newly-hatched nymphs. Only ticks which had been feeding on the cattle were found infected. It is evident that the infection of the eggs, and the supposed hereditary infection of offspring hatching from the eggs, has not been demonstrated for *C. hyalommæ* nor any of the other flagellates mentioned above.

Crithidia euryophthalmi McCulloch, 1917.—This flagellate is parasitic in the gut of the bug *Euryophthalmus convivus*, which feeds on the plant *Lupinus arboreus*, growing in sand dunes near San Francisco (Fig. 168). It was discovered by McCulloch (1917), who has given an account of its life history, which is of interest in that two phases of development not hitherto recorded in the life-history of these flagellates are described. These are multiple segmentation and internal budding. The alimentary tract of the bug consists of fore-, mid-, and hind-gut (Fig. 168). The fore-gut is made up of the mouth, pharynx, œsophagus, and proventriculus; the mid-gut of the crop, mid-stomach, pyloric expansion, and intestine; and the hind-gut of the colon, into which open the Malpighian tubes and the rectum. The type of flagellate found varies with the position in the gut. The œsophagus and proventriculus have always been found free from infection, the hind-gut has shown a slight infection in the rectum in some instances, while it was in the mid-gut that the heavy infections occurred. The stages which usually occur in the hindgut of insects are in this bug found in the pyloric expansion.

The forms which occur in the stomach (crop, mid-stomach, and pyloric expansion) are:

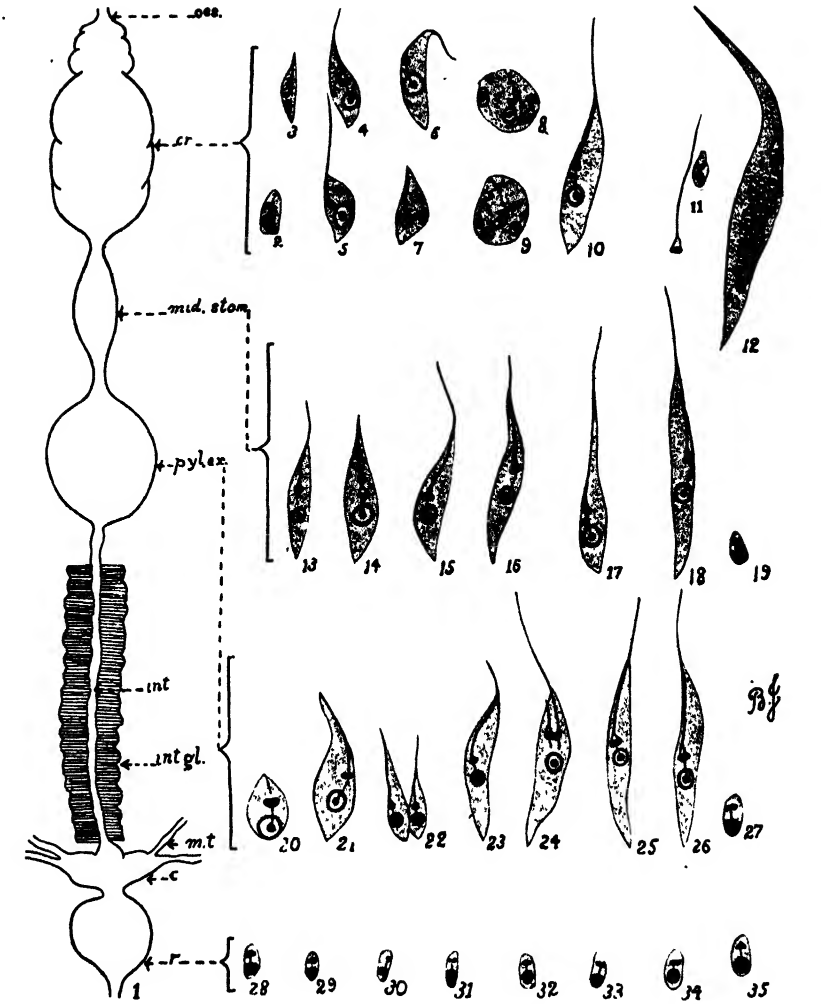


FIG. 168.—*Crithidia euryophthalmi* FROM INTESTINE OF *Euryophthalmus convivus*, ARRANGED SO AS TO SHOW THE VARIOUS FORMS WHICH OCCUR IN DIFFERENT PARTS OF THE ALIMENTARY CANAL (× 1,750). (AFTER MCCULLOCH, 1917.)

1. Diagram of alimentary canal: *œs.*, œsophagus; *cr.*, crop; *mid.stom.*, mid-stomach; *pyl.ex.*, pyloric expansion; *int.*, intestine; *int.gl.*, intestinal glandular epithelium; *m.t.*, Malpighian tubes; *c.*, colon; *r.*, rectum. 2-35. Various flagellate types explained in text.

1. Ovoid forms, which are presumably those taken up casually in the food. They are about 3·2 microns in length, and occur in the crop (Fig. 168, 2).

2. Every stage in growth of the ovoid forms up to the elongate flagellates 10 to 30 microns in length. As growth takes place, they migrate back-

wards from the crop to the mid-stomach and pyloric expansion (Fig. 168, 2–7 and 13–18).

3. Multiple division forms, which resemble the intracellular stages of development of *Trypanosoma lewisi* in the flea (Fig. 200, 9). They occur in the crop, and are presumably produced by growth associated with nuclear multiplication of flagellates which have entered the lining cells, and which by segmentation give rise to a number of flagellates corresponding with the number of nuclei (Fig. 168, 8–9).

4. Forms which are supposed to show a process of internal budding (Fig. 168, 12).

5. Binary fission forms of the usual type occurring in the crop and pyloric expansion (Fig. 168, 7).

6. Crithidia stages from the crop, which become free forms (nectomonads) in the mid-stomach and pyloric expansion (Fig. 168, 15–18).

7. Crithidia stages from the crop, which become attached forms (haptomonads) in the mid-stomach and pyloric expansion (Fig. 168, 13–14).

8. Final ovoid stages, which occur both in the mid-stomach and pyloric expansion. They are formed by a process the converse of that which occurred in the crop when the ovoid forms grew into the elongate crithidia forms. They become encysted, and pass back to the rectum as infective forms, to be passed in the fæces (Fig. 168, 19).

The interesting feature of this infection is that the cycle takes place comparatively far forwards in the gut, the final stages occurring in the pyloric expansion of the stomach, and not in the hind-gut. The ovoid encysted forms taken into the crop grow into the crithidia forms, which reproduce in the usual manner by longitudinal division, by an intracellular multiple segmentation, and by the curious internal bud formation. The latter is quite unique, and has not been described by any other observer (see p. 338). The crithidia forms produced in the crop pass back to the mid-stomach and pyloric expansion, where they may attach themselves to the lining epithelium or remain free. In either case, they retrogress to form the small ovoid encysting bodies. The various stages are illustrated in the diagram given by McCulloch, but confirmation of the intracellular stages and the internal budding process is required before they can be finally accepted.

Genus: Herpetomonas Kent, 1880.

The genus *Herpetomonas* was created by Kent (1880) for a flagellate of the house fly (*Musca domestica*), which was first mentioned by Burnett (1851, 1852) under the name *Bodo*. The next record of the fly flagellate is

that of Leidy (1856), who said he had frequently found *Bodo muscarum* in the intestine of the house fly in immense quantity. Later Stein (1878) referred to it as *Cercomonas muscæ domesticæ*, and gives *Bodo muscæ domesticæ* (Burnett) as a synonym, though, as noted above, Burnett referred to it only as *Bodo*. Finally, Kent (1880) referred it to his genus *Herpetomonas*, and called it *H. muscæ domesticæ* (Burnett), though this specific name was really Stein's. It would seem, therefore, as pointed out by Hoare (1924), that the correct name should be *H. muscarum* Leidy, 1856, as there is no doubt that Leidy and Burnett were both observing this flagellate, in spite of the fact that Becker (1923*c*) considers it a *nomen nudum*. Grassi (1879*a*) referred to the flagellate as *Schedoacercomonas muscæ domesticæ*, and in 1882 as *Monomita muscarum*. The majority of observers refer to the organism as *Herpetomonas muscæ domesticæ*.

The members of this genus, as defined in this work, have not only leptomonas and crithidia forms in their cycle of development, but also trypanosomes forms. They are, nevertheless, purely invertebrate parasites, which pass from host to host in the encysted stage. In the writer's opinion, the bulk of evidence is in favour of the view that the flagellate of the house fly has a trypanosome stage occasionally, though it is most usually seen in the leptomonas form. If it should be demonstrated that the trypanosome forms which occur in the house fly in reality belong to a distinct species of flagellate, then the generic name *Herpetomonas* cannot be employed for the genus as here defined, and it will become a synonym of *Leptomonas*. In this case, probably Patton's name *Rhynchoidomonas* (p. 374) would have to be employed. Whether the name stands or not, it is an undoubted fact that there are many insect flagellates which conform to the definition of the genus *Herpetomonas* as given here, and which was emended in this sense by the writer (1913). The recent work of Drbohlav (1925), who has obtained cultures of the flagellate of *Lucilia cæsar*, affords a direct confirmation of the conclusions reached here. He informs the writer that cultures commenced with a single organism showed not only leptomonas, but also trypanosome forms. With these cultures specially bred *Musca domestica*, as well as *Fannia regina* and *L. sericata*, were infected. It may be concluded, therefore, that the flagellate of the house fly is identical with that of *L. cæsar*, and has both leptomonas and trypanosome stages in its life-history.

Herpetomonas muscarum (Leidy, 1856).—This flagellate is very common in the intestine of the house fly, *Musca domestica*, in all parts of the world (Figs. 159 and 169). In some localities, especially in the tropics, practically every fly examined is found to be infected. It has been described from a variety of hosts other than *Musca domestica*, but from

the work of Chatton and his collaborators (1911-1913) on the parasites of species of *Drosophila*, and that of Patton (1912*b*) on *Musca nebulo* and *Lucilia serenissima*, it appeared at one time that the specificity of the

FIG. 169.—*Herpetomonas muscarum* FROM THE INTESTINE OF THE HOUSE FLY, FIXED IN SCHAUDINN'S FLUID AND STAINED WITH IRON HÆMATOXYLIN (× 2,000). (ORIGINAL.)

1-3. Short forms with single flagellum.
4-9. Dividing forms, showing formation of new flagella as new outgrowth and division of kinetoplast and nucleus.

insect flagellates for their hosts was greater than has been supposed, and that it was possible that many of the flagellates which had been regarded as *H. muscarum* were in reality distinct species. Patton (1921), however,

as a result of further observations, states that he has found this flagellate in Madras in the following hosts: *M. nebulo*, *M. humilis*, *Fannia canicularis*, *Borborus* sp., *Drosophila* sp., *Lucilia argyricephala*, *L. craggi*; while Becker (1923*d*) has shown by actual cross-infection experiments that in North America it may infect *Phormia regina*, *Lucilia sericata*, *Calliphora erythrocephala*, *Cochliomyia* (*Chrysomyia*) *macellaria*, *Musca domestica*, and *Sarcophaga bullata*. He believes that *H. muscarum*, *H. luciliæ*, *H. calliphoræ*, *H. sarcophagæ*, and the *Herpetomonas* which occurs naturally in *P. regina* and *C. macellaria*, belong to one species, *H. muscarum*. The similar results obtained by Drbohlav (1925) have been referred to above (p. 364).

As regards the distribution of *H. muscarum* in the fly, it may occur in any part of the gut up to the opening of the proventriculus. In the fully-grown leptomonas form it has a pointed, blade-like body up to 30 microns in length and 2 to 3 microns in breadth. The flagellum is often three times the length of the body. The nucleus is central in position, and the elongated kinetoplast is near the anterior end, and consists of the usual parabasal body, and the blepharoplast from which the axoneme of the flagellum arises. The anterior end of the body is often truncated or cut off, and a clear area may sometimes be seen to run into the cytoplasm towards the kinetoplast. In some cases this clear, funnel-like area appears to be continued past the kinetoplast, where it terminates indefinitely in the cytoplasm. The fact that in some individuals structures like bacteria were seen at the posterior end of the body led the writer (1913*a*) to suggest that this structure might be of the nature of a cytostome. This, however, seems very doubtful, for Becker (1923*c*) could detect no cytostome. It is found not only in the adult leptomonas forms, but also in the shorter and broader types on the way to encystment.

A feature of this flagellate, which has given rise to some controversy, is the frequent occurrence of two flagella (Fig. 169). This fact led Prowazek (1904) to define the genus *Herpetomonas* as including biflagellate organisms. The observations of Patton (1908*b*), Porter (1909*b*), Mackinnon (1910), and the writer (1911*a*) have clearly shown that the biflagellate individuals, which may comprise the majority of forms seen in an infection, are in reality dividing forms (Fig. 159). When division is proceeding, the blepharoplast elongates transversely, and a new axoneme growing out of a new flagellum appears even before division of the blepharoplast is completed. By the time the blepharoplast has divided, the new flagellum may be as long, or nearly as long, as the original one. The daughter blepharoplasts may proceed to division again, with a new axoneme forming from each one, and this may occur before the parabasal or the nucleus

has completed the first division. In this manner, organisms with four flagella and a single dividing nucleus may appear, and give the impression of a dividing biflagellate organism. A similar condition is sometimes seen in trypanosomes dividing actively in the blood of inoculated rats, where large forms may occur with four nuclei, four kinetoplasts, and four membranes and flagella. That the explanation given of the biflagellate appearance is the correct one is borne out by the fact that in flies, where active multiplication is not in progress, the flagellate has only a single flagellum.

It is in the leptomonas form that the flagellate is most commonly seen in flies. As pointed out by the writer (1913*a*), the kinetoplast may change

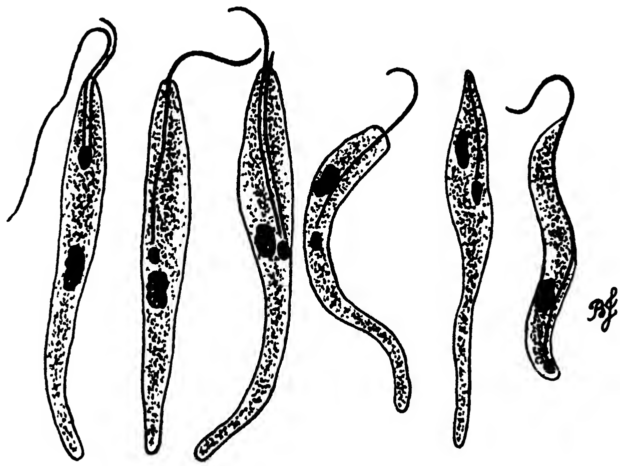


FIG. 170.—*Herpetomonas muscarum* OF HOUSE FLY ($\times$ *ca.* 2,000): TRANSFORMATION OF LEPTOMONAS INTO TRYPANOSOME FORMS. (AFTER WENYON, 1913.)

its anterior position for one near the nucleus, in which case the axoneme passes along the surface of the body (Fig. 170). Such forms have the crithidia structure, though an undulating membrane, as a definite band of cytoplasm, is not actually present. With further migration backwards of the kinetoplast, trypanosome forms are produced. The conditions under which this takes place are not known. The occurrence of these three phases has been noted in many allied flagellates. Some observers believe they represent distinct species, but the bulk of evidence is in favour of regarding all the forms as belonging to the cycle of the one flagellate. Fig. 170 shows the various transition forms in an infection where the leptomonas and the trypanosome types both occur (see also Fig. 155). Rosenbusch (1909) noted these different forms in the flagellate of the house fly, which, on this account, he termed *Crithidia muscæ domesticæ*. Becker (1923*c*) has confirmed these observations, while the

culture experiments of Drbohlav (1925), referred to above (p. 364), appear to be conclusive.

By a gradual shortening of the body of both the leptomonas and trypanosome forms, smaller stumpy individuals are produced, and these become attached to the lining epithelium of the hind-gut. Reproduction of all these free and attached forms takes place by longitudinal fission, often producing enormous infections of the gut. Encystment takes place by a gradual shrinkage of the body, or in some of the trypanosome individuals by the doubling of the body into a U, the space between the limbs of which gradually fill in, so that the axoneme follows a characteristic curved course in the cytoplasm. There are three methods

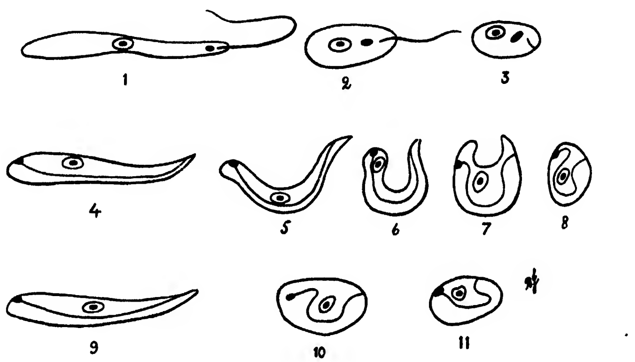


FIG. 171.—THREE METHODS BY WHICH THE ROUNDING-UP (ENCYSTMENT) OF *Herpetomonas muscarum* TAKES PLACE IN THE HIND-GUT OF THE HOUSE FLY. (AFTER WENYON, 1913.)

1-3. Retraction of leptomonas form.
4-8. Rounding up of trypanosome form by looping of the body.
9-11. Rounding-up of trypanosome form by retraction of body.

by which retraction of the body and encystment may take place (Fig. 171). Becker (1923*c*) thinks that encystment of *H. muscarum* always takes place after the trypanosome type has been developed. The cysts appear to have a definite cyst wall. Their function is undoubtedly the transmission of infection from fly to fly, but, as Patton (1910*b*) and Becker (1923*c*) have shown, flies may be infected by ingestion of adult flagellates or the pre-encysting forms passed in the fæces, as well as by cysts. The cysts would probably ensure protection against a period of desiccation.

From his earlier work, Patton concluded that, though the larvæ of *Musca nebulo* might be infected with flagellates, these did not appear to survive the pupal stage, as flies hatched from infected larvæ were free from infection. In a later paper (1921) he appears to have modified this view, for he states that infections will pass through the pupæ to the adults. Becker (1923*c*) was unable to detect larval infections. As

already pointed out, *H. muscarum*, like the flagellates considered above, passes from insect to insect in cysts voided in the fæces by infected individuals. It differs from *L. ctenocephali* and *C. gerridis* in that the flagellate may assume the trypanosome form in the course of its developmental cycle.

Patton (1921) records the successful culture of *H. muscarum* in N.N.N. medium. The strain was obtained by dissecting out the peritrophic membrane of a *Lucilia argyricephala*, and inoculating the medium with some of the contained flagellates. Glaser (1922), who also succeeded in cultivating the organism, has shown that grasshoppers can be infected by inoculation into the body cavity. The cultures obtained by Drbohlav have been noted above (p. 364).

Franchini and Mantovani (1915), and Fantham (1922), claim to have infected rats and mice with *H. muscarum*. Glaser (1922) and Becker (1923*a*) have been unable to confirm this observation.

OTHER MEMBERS OF THE GENERA LEPTOMONAS, CRITHIDIA, HERPETOMONAS.

As will be seen from the list (p. 1402), the number of invertebrate flagellates is very great, but in the majority of cases nothing like a complete cycle has been observed. In some of those where it is known, as, for example, *L. ctenocephali*, *L. culicis*, *L. jaculum*, *C. pulicis*, *C. gerridis*, etc., the cycle of development is a comparatively simple one, the encysted forms ingested growing through the pre-flagellate form into the adult flagellate, and then retrogressing through a post-flagellate form into the cyst, which escapes in the fæces and is ingested by a new host. In most cases the feeding habits of the adults, as the house fly, are such that infection by the ingestion of cysts is possible. In other cases, as, for instance, fleas and sand flies, the adults are blood feeders, which have no opportunity of ingesting cysts. There are, however, larval stages, which are omnivorous feeders, and the adults become infected during metamorphosis from the infected larvæ. Other blood suckers have no stage capable of ingesting cysts, and it would appear that infection can be derived only from the blood. The crithidial infection of *Hyalomma ægyptium*, mentioned above, is of this type. The recent discovery of a *Leptomonas* in the proboscis and intestine of *Glossina morsitans* by Lloyd (1924) affords another instance of the same type. The origin of this *Leptomonas* is not known, but if it conforms with other flagellates of tsetse flies, it must have originated from the blood of some vertebrate. It may be connected with the leishmania infections of man which occur in Nigeria, or be derived from the blood of a reptile on which these flies readily feed.

Forms found in the Body Cavity and Salivary Glands.

The infections are in most cases purely intestinal ones, though the flagellates may sometimes find their way into the Malpighian tubes. Occasionally, however, the infection extends from the gut to the body cavity. Zotta (1912 and 1921) described a flagellate infection of *Pyrrhocoris aptera*, a plant bug. The organism (*L. pyrrhocoris*) occurred, not only in the gut, but also in the body cavity and salivary glands, as again noted by Franchini (1922*b*). *C. hyalommæ*, described above, is peculiar in that it occurs in the body cavity of the tick, whence it infects all the tissues of the body, including the salivary glands, but is absent from the intestine.

Robertson (1912) found flagellates of the leptomonas type in the salivary glands of a plant bug (*Leptoglossus membranaceus*), and Hollande (1912) found a form, which was named by him *L. emphyti*, in the hæmocœle fluid of a hymenopteran larva (*Emphytus cinctus*), a mere puncture of the cuticle yielding a fluid teeming with flagellates. Glasgow (1914) noted a salivary gland infection in another bug (*Peribalus limbolarius*). Working with pentatomid bugs (*Pentatoma ornata* and *P. juniperina*), Franchini (1922*b*) stated that not infrequently the crithidia, which inhabited the intestine, invaded the salivary glands. Poisson (1925) has noted that *L. naucoridis* of the water bug *Naucoris maculatus*, though usually confined to the intestine, may, in the case of heavy infections, invade the body cavity and internal organs, including the salivary glands. While dissecting a species of *Culex* in Tonkin, Mathis (1914) noted infection of the salivary glands with a flagellate of the crithidia type. In the case of the plant bugs, it is possible that the flagellates may be developmental stages of some plant parasite, while those in the tick and mosquito may have been derived from vertebrate trypanosomes.

Roubaud's Genus Cercoplasma.

While in the Congo, Roubaud discovered a remarkable flagellate in the intestine of *Pycnosoma putorium*. He (1908) named it *L. mirabilis*, but subsequently (1911) created the new genus *Cercoplasma*, in which he placed it, together with other similar forms he had found. It will be seen that, apart from certain large giant individuals, the flagellate shows the usual types of the genus *Herpetomonas*, and in view of the fact that some of the flagellates ascribed by Roubaud to his genus *Cercoplasma* lack these giant forms, they will be regarded as belonging to the genus *Herpetomonas*. Roubaud's flagellate then becomes *H. mirabilis* (Roubaud, 1908). The main features of the flagellate are shown in Fig. 172. The giant forms may exceed 200 microns in length, with a maximum breadth

of 3·5 to 5 microns. The leptomonas and trypanosome forms are of the usual dimensions, being 18 to 20 microns in length, with a flagellum up to twice the length of the body. All intermediate stages between these small forms and the giants occurred. In the rectum, small forms, 4 to 10 microns in length, were found. These were evidently encysting forms. It is difficult to account for the giant forms, which have been seen only in this and the allied flagellates, *H. mesnili* and *H. lineata*, though smaller forms of the same type occur in Chatton's *H. roubaudi* described below (Fig. 176). It appears to be not improbable that they are merely abnormal overgrowth forms, in which, for some reason, nuclear division has been delayed, allowing a great increase in the cytoplasm to take place, as occurs

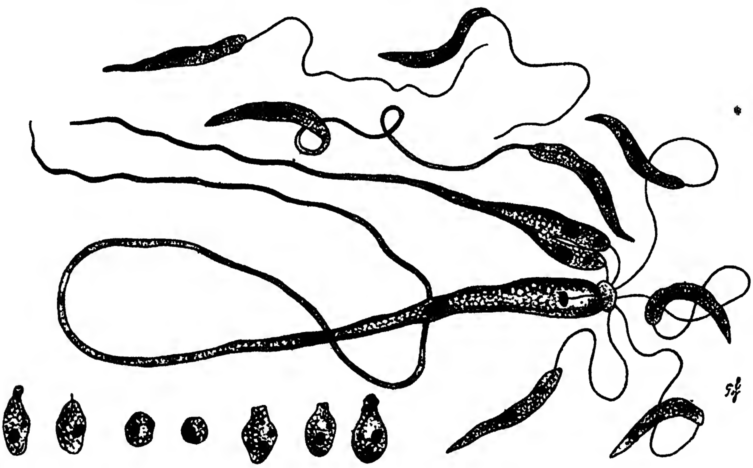


FIG. 172.—*Herpetomonas mirabilis* FROM *Pycnosoma putorium*, SHOWING VARIOUS TRYPANOSOME, LEPTOMONAS, ELONGATE CERCOPLASMA, AND ROUNDED FORMS (× 900). (AFTER ROUBAUD, 1909.)

in the case of *Trypanosoma rotatorium* of frogs, *Trichomonas vaginalis*, and other flagellates.

Herpetomonas mesnili (Roubaud, 1908) was first called *Leptomonas mesnili* by Roubaud (1908*b*), and later included in his genus *Cercoplasma* (1911*a*). It is a parasite of *Lucilia latifrons*, and *Lucilia* sp. of the Congo. Both this parasite and *H. mirabilis* were later found by Roubaud in a species of *Pycnosoma* and *Lucilia* in the French Sudan. In *H. mesnili*, the giants are not more than 70 microns in length, while the small forms vary from 7 to 8 microns and upwards, and have a flagellum from 12 to 14 microns in length. Round and encysting forms are not described. The flagellate was only seen twice in the Congo—once in a fly in pure culture, and once in association with two other flagellates, one morphologically a crithidia and the other a leptomonas.

Patton (1921) reports having found *H. mirabilis* in various flies in Madras. He has seen it in *Lucilia argyricephala*, *L. craggii*, *Chrysomyia* (*Pycnosoma*) *megalocephala*, and *C. albiceps* (*Pycnosoma putorium*). The cycle of development in *L. argyricephala* is described as follows. In the larvæ the growth of rounded leishmania forms into leptomonas forms and finally into the elongate cercoplasma forms was noted. The flagellates persist in the pupæ, and appear in the adult flies. Here in the hind-gut the leptomonas forms become transformed into flagellates of the trypanosome type. The nucleus becomes elongated, while the kinetoplast passes backwards to a point near the posterior end of the body. The trypanosome stage having been reached, the flagellates become shorter, and, finally, rounded leishmania or post-flagellate forms are produced. It is by ingestion of these that the larvæ and, presumably, the adults become infected. It is possible that this flagellate represents one of the phases of development of *H. muscarum*.

Another flagellate, which Roubaud places in his genus *Cercoplasma*, is *Herpetomonas caulleryi* (Roubaud, 1911). This was found in *Auchmeromyia luteola* in the French Sudan. It agrees with the two forms *H. mirabilis* and *H. mesnili* as regards the various small forms, but the giant forms were not seen. The flagellate discovered by Roubaud (1912*c*) in a species of *Drosophila* in the French Sudan, and named by him *Cercoplasma drosophilæ*, is probably identical with one of the species of *Herpetomonas* described by Chatton from *D. confusa* (see below). All these flagellates were limited to the intestinal tract of the flies.

Swingle (1911) has described large giant forms of *Herpetomonas lineata* of *Sacrophaga sarraceniæ* in North America. In this case the longest forms may even reach a length of 385 microns, and, like Roubaud's giants, there is a swollen anterior end containing nucleus and kinetoplast, and a very long drawn-out post-nuclear region. The flagellum is short, a fact which would suggest that they are normally attached to the gut epithelium. As in *H. mirabilis*, the giant leptomonas forms were associated with flagellates of the trypanosome and other types. This form, again, may be a phase of *H. muscarum*.

Roubaud's Genus Cystotrypanosoma.

The genus *Cystotrypanosoma* was proposed by Roubaud (1911) for flagellates which have the trypanosome structure, and which produce cysts in the rectum of the flies. Of this type is a flagellate he found in the intestine of a species of *Lucilia*, probably *L. sericata*, at Bamako in the French Sudan. Ordinary trypanosome forms occur which in the rectum become smaller and doubled as a U, and by a fusion of the limbs there is produced an ovoid body which encysts. Larger forms of the rhyn-

choidomonas (see below) type of Patton were also found. According to Roubaud, it has been the custom to include in the genus *Trypanosoma* the typical forms parasitic in the blood of vertebrates, and those which are purely insect flagellates. The latter produce cysts, as noted above, while the former do not. Accordingly, he proposes to divide the genus *Trypanosoma* into two sub-genera: *Trypanosoma* for the blood parasites

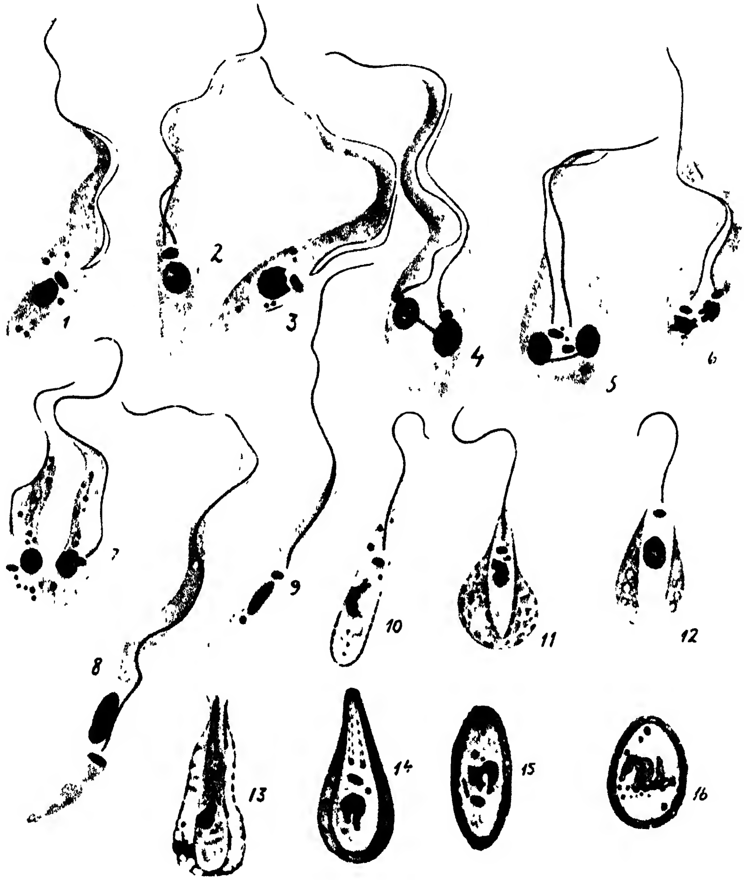


FIG. 173.—*Trypanosoma grayi* IN INTESTINE OF *Glossina palpalis* (× 2,000). (AFTER MINCHIN, 1908.)

1-7. Division of typical crithidia forms. 8. Trypanosome form. 9-16. Stages in the supposed formation of the cyst.

of vertebrates, and *Cystotrypanosoma* for the cyst-producing forms of invertebrates. Another flagellate, which Roubaud includes in the latter genus, is *Herpetomonas grayi* Novy, 1906 (called by Novy, Minchin, and others *Trypanosoma grayi*, and by Roubaud *Crithidia grayi*). This organism (Fig. 173) occurs in the digestive tract of *Glossina palpalis*, *G. brevipalpis*,

G. fusca, and *G. tachinoides*, where it may give rise to confusion with developmental stages of *T. gambiense* and other trypanosomes. It occurs in the trypanosome form as well as others. and cysts are said to be produced in the rectum. Kleine and Taute (1911), and Kleine (1919*a*), working with bred flies which were fed on a known infected crocodile, have demonstrated that *H. grayi* is in reality the developmental form of *T. kochi*, the crocodile trypanosome; while Lloyd, Johnson, Young, and Morrison (1924) have shown that laboratory bred *G. tachinoides* become infected with *H. grayi*, or flagellates indistinguishable from it, after feeding on monitors (*Varanus exanthematicus*) harbouring *T. varani*, or on toads (*Bufo regularis*) harbouring a trypanosome resembling *T. varani*, as well as on crocodiles. It appears, therefore, that *H. grayi* represents the invertebrate phase of a trypanosome, so that the alleged presence of encysted forms in the rectum of the flies requires an explanation, as these stages are not known to occur in the case of any other trypanosome. Fraser and Duke (1912*b*) failed to cause laboratory bred flies to infect themselves from the fæces of infected flies. As explained above (p. 342), it seems probable that the supposed cysts of *H. grayi* are not actually of this nature. Minchin, Gray, and Tulloch (1906), and Minchin (1908) suggested that *H. grayi* might be a bird trypanosome. Of this there is at present no direct evidence.

The genus *Cystotrypanosoma*, as defined by Roubaud, corresponds with the genus *Herpetomonas* as it is interpreted in this work. As members of Roubaud's genus *Cercoplasma* produce cysts, the distinction between it and the genus *Cystotrypanosoma* is not very clear.

Patton's Genus Rhynchoidomonas.

Patton (1910*a*) described a flagellate which he had found in the Malpighian tubes of *Lucilia serenissima* of Madras, and which appeared to differ from the well-recognized types. He described it under the generic name of *Rhynchomonas*, but as this was pre-occupied, later in the same year he substituted the name *Rhynchoidomonas* (Fig. 174). The flagellates were only seen in a single fly. Flagellates of the same type were seen by the writer (1911) in the gut and Malpighian tubes of house flies in Bagdad. These flagellates were also seen by Alexeieff (1911) in species of *Calliphora* and *Lucilia* in Europe (Fig. 155), and by Patton (1921) in another fly in Madras.

In the writer's experience (1911*a*) they occurred in association with *H. muscarum*, and it was concluded that they represented developmental stages of this common house-fly flagellate. Later, in Aleppo, the question was again studied by the writer, and the view was adopted that the trypanosome forms actually belonged to the cycle of *H. muscarum*, as every stage in the migration backwards of the nucleus could be traced.

Dunkerly (1911) and Alexeieff (1911*f*, 1912*e*) also regarded these forms as representing developmental stages of *H. muscarum*, which, however, is most usually met with in the leptomonas form. Chatton (1913) expressed the opinion that the flagellate, *H.* sp. (1) referred to below (p. 378), of the Malpighian tubes of the adult *Drosophila confusa* had been evolved from the intestinal species. Hence, he suggests that these Malpighian tube forms should be placed in a distinct genus for which Patton's name has priority. The type species of this genus, according to Chatton, will be

FIG. 174.--LIFE-CYCLE OF *Rhynchoidomonas siphunculinæ* IN INTESTINE OF *Siphunculina funicola* (× *ca.* 2,000). (AFTER PATTON, 1921.)

1-2. Pre-flagellate forms from stomach of fly.
3-5. Growth of flagellate and formation of flagellum in Malpighian tubes.
6-9. Development of fully-formed flagellate of Rhynchoidomonas type in Malpighian tubes.
10-13. Retraction of flagellate forms towards the post-flagellate stage in Malpighian tubes.
14. Post-flagellate stages which escape from the Malpighian tubes into the intestine and are passed in the fæces.

R. drosophilæ [*H.* sp. (1)], which is said to occur only in the Malpighian tubes of *D. confusa.* As many insect flagellates are known to occur in the intestine, and occasionally in the Malpighian tubes (*e.g.*, *L. ctenocephali* of the flea), it cannot be considered as finally established that the Malpighian tube forms are distinct from the intestinal ones.

Patton (1921) has given an account of what he regards as the complete life-cycle of one of these forms, which he names *R. siphunculinæ*, and

which occurs in the Malpighian tubes of the eye fly, *Siphunculina funicola*, of Madras (Fig. 174). The life-cycle which he describes follows closely those of other arthropod flagellates studied by him (Fig. 166). The pre-flagellate stage resulting from ingested cysts occurs in the stomach of the fly, but, unlike the pre-flagellate forms of species of leptomonas and crithidia, these do not reproduce, but merely increase in size, while the axoneme becomes evident. Further growth takes place only in the Malpighian tubes, where the typical rhynchoidomonas forms are produced. When fully formed, these may measure 55 microns in length. The nucleus lies nearer the anterior than the posterior end, and the kinetoplast lies near but posterior to the nucleus. From the kinetoplast the axoneme passes to the anterior end along the surface of the body. A definite undulating membrane is not developed, nor is the axoneme continued beyond the anterior extremity of the body. The part of the flagellate behind the nucleus varies considerably in length according to the stage of development. At first it is quite short, but in the fully-formed flagellates it may be drawn out into a long, tapering, cytoplasmic process three or four times as long as the portion of the body anterior to the nucleus. Multiplication takes place by longitudinal division after division of the kinetoplast and nucleus, but, contrary to what usually occurs in other Trypanosomidæ, the body commences to divide at the posterior extremity. After the flagellate stage has been reached, development towards the post-flagellate takes place. The long posterior portion of the body is withdrawn, and forms very much like pre-flagellate stages arise. In some of these the kinetoplast is near the posterior extremity of the body, and the nucleus nearer the anterior end. These forms are attached to the cells of the Malpighian tubes in clusters. Eventually, small rounded or oat-shaped forms are developed, and these escape into the gut and are excreted with the fæces. Patton was able to demonstrate that this flagellate never developed in the larvæ of the fly which were fed on the dead bodies of adult flies harbouring this parasite. On the other hand, another form (*Herpetomonas siphunculinæ*), which occurred in the intestine of the fly, readily infected the larvæ and appeared in the intestine of the adult. The fact that typical trypanosome forms with free flagella occurred in the cycle of the *H. siphunculinæ* in the intestine of the fly, and that this flagellate was never found in the Malpighian tubes, raises the question of its relationship to the rhynchoidomonas form, which may be a stage of evolution of *H. siphunculinæ*. The peculiar features of its morphology may be due to the fact that it has invaded the Malpighian tubes, which is not its usual habitat. The writer cannot agree with Patton that the rhynchoidomonas forms are not of the trypanosome type. It is known that in typical trypanosomes, as, for instance, *T. lewisi*

the post-nuclear region of the body may be extremely long. A similar though more marked hypertrophy occurs in Roubaud's *H. mirabilis.* Furthermore, in typical trypanosomes the width of the undulating membrane varies considerably, so that in some forms the axoneme appears to pass along the surface of the body, as in the rhynchoidomonas forms here under discussion, while it is well known that in many forms of trypanosome no flagellum exists. If these variations were combined in one individual, and the kinetoplast brought nearer the nucleus, then the characteristic rhynchoidomonas form would be produced. As a matter of fact, in some of the forms depicted by Patton the kinetoplast is far behind the nucleus, so that in all essential respects the rhynchoidomonas forms are of the trypanosome type, and the flagellate will be considered here as belonging to the genus *Herpetomonas.* The fact that the axoneme does not extend beyond the anterior end of the body probably indicates that these rhynchoidomonas forms are really attached forms. Furthermore, this fact may explain the commencement of division at the posterior unattached end of the body instead of at the attached anterior end, where it usually occurs.

Chatton's Observations on the Trypanosomidæ of Drosophila.

In certain cases, as appears chiefly from the work of Chatton and his colleagues on the flagellate parasites of various species of *Drosophila,* the cycle of development may not be so simple as in the forms described above. In *Drosophila confusa* he has been able to identify four, or possibly five, distinct species as a result of extensive breeding experiments extending over several years. He has succeeded in separating the flagellates, and has obtained them in pure culture in different batches of the fly.

In order to comprehend properly Chatton's views, it will be necessary to describe a structure which occurs both in the larvæ and adults of the *Drosophila* (Fig. 175). This is the peritrophic membrane which arises at the œsophageal opening of the stomach as a cylinder and passes back through the stomach to end in the hind-gut. The anterior end of this membrane is attached as the diagram shows, but the posterior end is lying free in the gut cavity. It is a membranous structure, possibly of a chitinous nature, and, as far as can be seen, is not perforated, so that organisms cannot pass through it. The lumen of the cylinder is the endotrophic space, while that between it and the gut lining epithelium is the peritrophic space. The function of the membrane is not properly understood, but it naturally suggests a filtration process in connection with nutrition.

Of the flagellates of *D. confusa, Leptomonas roubaudi* Chatton, 1912, is perhaps the simplest (Fig. 176). It has only been found in the Malpighian tubes of the larva and the adult, where it occurs in the various forms depicted. It will be seen that the elongate forms are leptomonas in type, and these gradually merge into trypanosome forms, which become round and finally encysted. According to the definition of genera adopted here, this parasite will be known as *Herpetomonas roubaudi.*

The second flagellate is *Trypanosoma drosophilæ* Chatton and Alilaire, 1908. It occurs in the larvæ, pupæ and adults of *D. confusa.* In the larvæ and pupæ it occurs only in the peritrophic space, while in the adult it occurs only in the Mal-

pighian tubes or in the peritrophic space near their openings. The flagellate occurs in the trypanosome form, which, still maintaining this structure, becomes a smaller trypanosome form. This becomes doubled into a U form in which the two limbs fuse, and the resulting body then encysts. This process is similar to that described above for *H. muscarum*. Here, again, the cycle is a simple one (monophasic), and as in the first-mentioned flagellate, by simple reduction in size and retraction of the body, the cyst is produced (Fig. 177). This flagellate, showing the trypano-

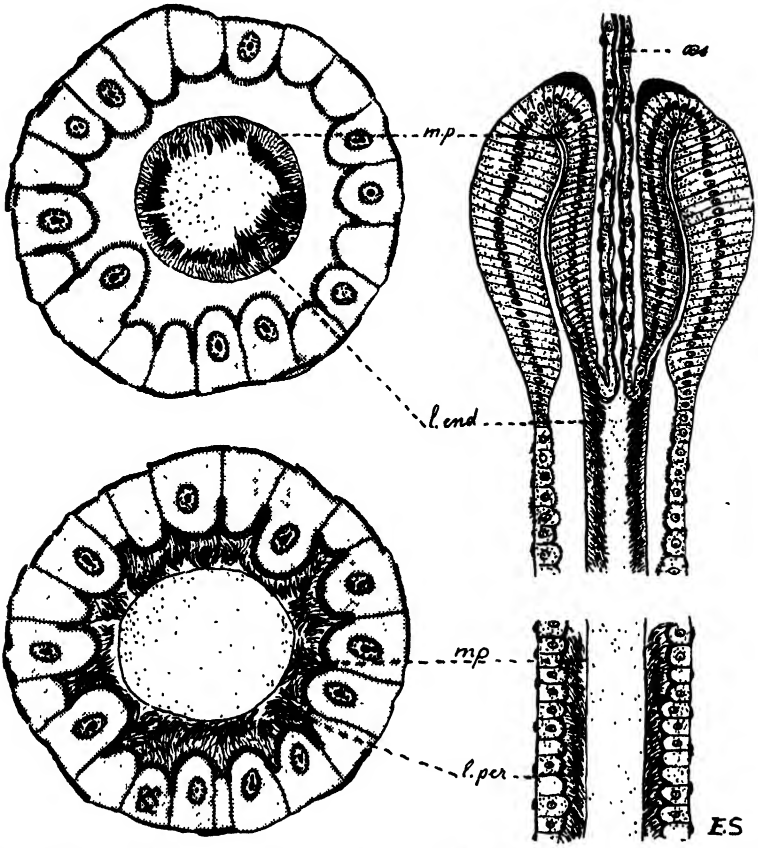


FIG. 175.—ARRANGEMENT OF THE PERITROPHIC MEMBRANE IN THE INTESTINE OF *Drosophila confusa*. (AFTER CHATTON AND LEGER, 1912.)

m.p, Peritrophic membrane; *œs*, œsophagus; *l.end*, flagellates in the endotrophic position; *l.per*, flagellates in the peritrophic position.

some form in its cycle, becomes *Herpetomonas* sp. (1). Chatton (1913) notes that the trypanosome forms which occur in the Malpighian tubes are related to the Rhynchoidomonas described by Patton. The third and fourth flagellates of this fly are closely related. They are described as *L. drosophilæ* by Chatton and Alilaire (1908), and *Leptomonas* sp. by Chatton and Leger, M. (1912*a*). The former occurs as an endotrophic infection in the larva and as a peritrophic infection in the adult, while the latter is only found in the adult in the endotrophic space. The first of these, *L. drosophilæ*, which will be called here *H. drosophilæ*, occurs in the adult fly in

various forms—trypanosome, crithidia, leptomonas, leishmania and cyst (Fig. 178). The leptomonas forms are regarded as the flagellates which develop directly from the cysts. By backward migration of the kinetoplast, the crithidia forms, and

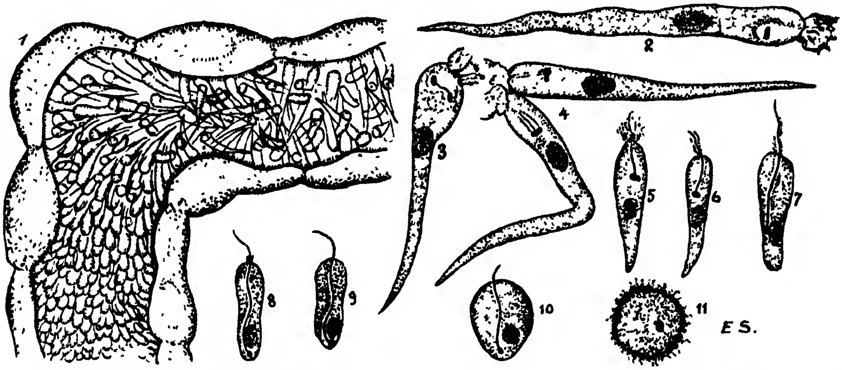


FIG. 176.—*Herpetomonas roubaudi* FROM MALPIGHIAN TUBES OF *Drosophila confusa* (× *ca.* 2,000). (AFTER CHATTON, 1912.)

1. Malpighian tube packed with flagellates.
3-4. Large attached forms of the cercoplasma type (gregarinien).
5-9. Transitions from the leptomonas (monadien) to the trypanosome form (spermoide).
10-11. Stages of encystment.

finally the trypanosome forms, are evolved. As the latter pass to the hind-gut, the kinetoplast comes forward again, and there are again produced leptomonas forms

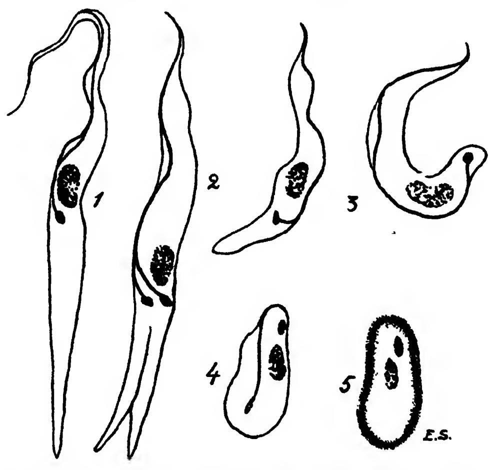


FIG. 177.—*Herpetomonas* sp. OF *Drosophila phalerata* (× *ca.* 2,000). (AFTER CHATTON AND LEGER, 1912.)

This flagellate is similar to *H.* sp. (1) of *D. confusa.*

1-2. Rhynchoidomonas forms. 3. Trypanosome forms.
4-5. Encystment after looping of body.

which attach themselves to the epithelial lining of the gut. Here they become still further retracted, till the round leishmania forms which produce the cysts result. Reproduction takes place in all these stages, and the reduction in size, leading to

cyst formation, is rather the result of successive divisions unassociated with growth than to actual retraction. In this cycle it will be seen that the leptomonas forms appear in two phases, so that, to use Chatton's term, the developmental cycle is diphasic in contrast to that of *H. roubaudi* and *H.* sp. (1) described above, in which it is monophasic.

There is a modified cycle of development exhibited by another peritrophic form often associated with *H. drosophilæ* which is monophasic. The free leptomonas forms, instead of becoming free trypanosome forms, as in *H. drosophilæ*, pass to the hind-gut as leptomonas forms, where they become smaller and attach themselves to the gut wall. At the same time the kinetoplast migrates backwards, so that the attached forms really have the trypanosome structure. This cycle corresponds closely with that of *Herpetomonas* sp. (1), the second flagellate mentioned above, and can be considered as a condensed cycle by the loss of the active trypanosome stage, which is only revealed after the leptomonas forms have attached themselves.

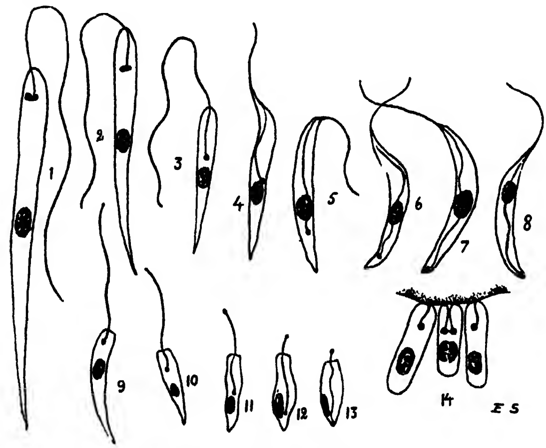


FIG. 178.—*Herpetomonas drosophilæ* FROM INTESTINE OF *Drosophila confusa* (× 2,000). (AFTER CHATTON AND LEGER, 1911.)

1-2. Leptomonas forms (monadien).
3-8. Transformation of leptomonas into trypanosome forms (trypanoide).
9-13. Transformation of leptomonas forms (monadien) into small ovoid bodies (spermoide).
14. Attached forms (gregarinien) in rectum which become encysted after becoming trypanosome forms (spermoide).

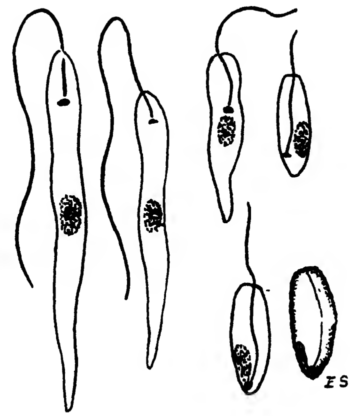


FIG. 179.—*Herpetomonas* sp. OF *Drosophila phalerata* (× *ca.* 2,000), FREE LEPTOMONAS AND TRANSFORMATION OF ATTACHED LEPTOMONAS FORMS (GREGARINIEN) INTO TRYPANOSOME FORM (SPERMOIDE) BEFORE ENCYSTMENT. (AFTER CHATTON AND LEGER, 1912.)

Chatton and Leger, however, speak of this flagellate as *Leptomonas* p., as they have not sufficient evidence, in the shape of pure infections in the fly, to justify separating it entirely from the diphasic form, *H. drosophilæ*. A flagellate of *D. phalerata* is, however, very similar to it (Fig. 179).

The fifth flagellate is *Leptomonas* sp. Chatton and Leger, 1912. It, again, is a *Herpetomonas* [*H.* sp. (2)], according to the definition adopted here, and differs from *H. drosophilæ* in being endotrophic and not peritrophic in the adult fly. It occurs only as an intestinal parasite of the adult fly. The forms met with are similar to those of *H. drosophilæ*, with the exception that reduction in size of the body takes place to a certain extent and then ceases, so that cysts are not formed. From

observations on the allied flagellates of *D. ampelophila* it would appear that these small forms, still provided with flagella, are found in the fæces, where they can readily be seen. In this endotrophic parasite, which has a diphasic cycle, cyst formation has so far not been discovered.

The flagellates described by Chatton and his co-workers from *D. confusa* can be tabulated as follows:

1. *H. roubaudi* (= *L. roubaudi* Chatton, 1912).—Malpighian tubes of larva and adult: monophasic cycle.

2. *H.* sp. (1) (= *T. drosophilæ* Chatton and Alilaire, 1908 = *Rhynchoidomonas drosophilæ* Chatton, 1913).—Larva (peritrophic), adult (Malpighian tubes): monophasic cycle.

3. *H. drosophilæ* (= *L. drosophilæ* Chatton and Alilaire, 1908).—Larva (endotrophic), adult (peritrophic): diphasic cycle.

4. *H.* p. (= *L.* p. Chatton and Leger, 1912).—Larva (endotrophic), adult (peritrophic): monophasic cycle. It appears that this may represent an alternative cycle of *H. drosophilæ*, a view which receives support from the later observations of Chatton and Aubertot (1924), mentioned below.

5. *H.* sp. (2) (= *L.* sp. Chatton and Leger, 1912).—Adult (endotrophic): diphasic cycle.

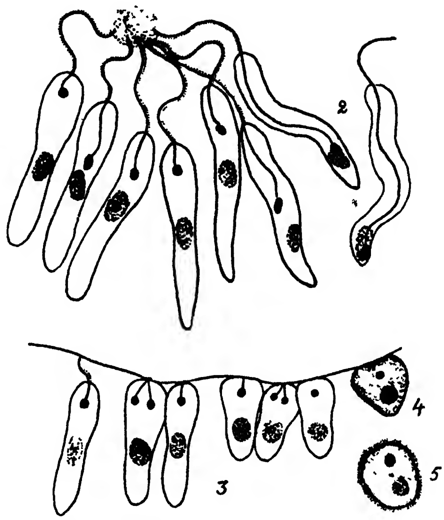


FIG. 180.—*Herpetomonas rubrostriatæ* OF *Drosophila rubrostriata* (× *ca.* 2,000). (AFTER CHATTON AND LEGER, 1912.)

1. Leptomonas forms (monadien).
2. Forms approaching the trypanosome type (trypanoide), which again become leptomonas forms (monadien).
3. Retracting forms attached to cells of hind-gut (gregarinien).
4-5. Encystment.

As many of the trypanosome forms of insect flagellates do not appear to have a well-developed membrane, the axoneme running either through the cytoplasm or attached directly to the surface of the body, Chatton employed the term *leptotrypanosome* to distinguish them from the trypanosomes (*eutrypanosome*), which are the typical vertebrate forms with a well-developed membrane. In later writings he employed the name *trypanoide* for the trypanosome forms of the insect flagellates. According to Chatton's nomenclature, the series of forms through which a flagellate may pass are these: *stade monadien* (leptomonas forms), which by backward migration of the kinetoplast becomes the *stade trypanoide* (trypanosome form).

The latter may revert to the *monadien* phase again. The *monadien* forms by shrinkage of the body become the short leptomonas forms, which attach themselves to the hind-gut epithelium. These Chatton terms *stades gregariniens*, and they by migration of the kinetoplast may assume the trypanosome arrangement, when they are known as *stades spermoides*. These latter forms become encysted. So that, in the diphasic cycle of *H. drosophilæ* and the allied *H. rubrostriatæ*, the following stages are passed through: monadien, trypanoide, monadien, gregarinien, spermoide, cyst (Fig. 178). The monophasic cycle of *H. roubaudi* (Fig. 176) is simpler: monadien, gregarinien, spermoide, cyst.

Chatton and his co-workers have devoted much time and trouble to the experimental side of this question, and though such a multiplicity of flagellates from a single host is somewhat disconcerting, his published results are difficult to explain on any other basis. Some of the flagellates have been kept in pure culture in a batch of flies for over two years, and, according to Chatton, the infections have always remained the same. Another, perhaps unexpected, result of his earlier work is that flagellates of nearly allied species seem to be specific to their hosts. Thus, working with three other species of *Drosophila*—viz., *D. rubrostriata*, *D. phalerata*, and *D. ampelophila*—it was found that when bred in captivity with infected *D. confusa* they did not acquire infection, though they themselves at other times are found to harbour flagellates which are difficult to distinguish from those of *D. confusa*. The flagellate of each host appeared to be specific for that host. As a result of his experiments, Chatton has named two of the flagellates, which become *H. rubrostriatæ* Chatton and Leger, 1911, and *H. ampelophilæ* Chatton and Leger, 1911 (Fig. 180). The flagellate of *D. rubrostriata* remained a pure peritrophic infection in a batch of flies from June, 1910, to March, 1911, during which time over 200 flies were examined. In these flagellates, both the diphasic and monophasic forms, like *H. drosophilæ* and *H.* p., occurred. On the other hand, a batch of *D. ampelophila* bred from June to December, 1910, always showed *H. ampelophilæ* as an endotrophic form, which corresponds to *H.* sp. (2) of *D. confusa*.

In a later publication Chatton and Aubertot (1924) modify the view regarding the specificity of the flagellate *H. drosophilæ* (= *L. drosophilæ* Chatton and Alilaire, 1908). In *D. confusa* it is always endotrophic in the larva, and both endotrophic and peritrophic in the adult. It has now been found that both larvæ and adults of *D. rubrostriata* can be infected with this flagellate. In both larvæ and adults the infection commences as an endotrophic one, but in the adult it may become peritrophic after a few days, owing to migration of the flagellates round the posterior free end of the peritrophic membrane. It follows that the flagellate *H. rubrostriatæ* may be identical with *H. drosophilæ*.

Genus: Phytomonas Donovan, 1909.

As explained above, the flagellates which are included in this genus have only the leptomonas and leishmania forms. A very good case for retaining them in the genus *Leptomonas* can be made, but as they occur in both plants and invertebrates, and sometimes in vertebrates also, if Strong's observations receive confirmation, they are conveniently placed in a separate genus like the forms included in *Leishmania* (Fig. 181). The name *Phytomonas*, suggested by Donovan (1909), will be employed.

Lafont (1909) described a flagellate of the leptomonas type as occurring in the latex of a plant, *Euphorbia pilulifera*, in the island of Mauritius. He named it *Leptomonas davidi*, and later rediscovered the organism in two other plants, *E. thymifolia* and *E. hypericifolia*. It is now known to occur in various parts of the world, as the table shows (p. 390). Various species of *Euphorbia* are involved, and it was supposed that flagellate infections were limited to plants of this family till Migone (1916) described an infection of *Araujia angustifolia* (*Funastrum boneoriensis*) in South America. Migone proposed the name *Leptomonas elmassiani* for the flagellate of

A. angustifolia, while França (1921) has given the name *L. bordasi* to another flagellate which, Migone informed him, he had found in a plant (*Morreira odorata*) belonging to the same family. Franchini (1922*c*, *f*, *k*) claims to have found flagellates of various kinds, not only in plants belonging to the Euphorbiaceæ, but in many others. França (1920*a*) has

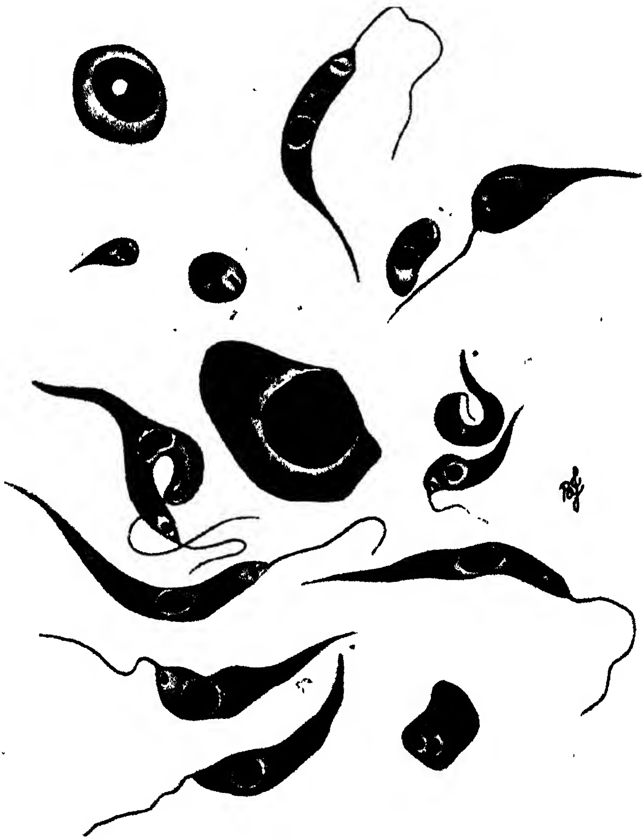


FIG. 181.—*Phytomonas davidi* IN THE JUICE OF AN INDIAN EUPHORBIA. (FROM DRAWINGS PRESENTED TO THE WRITER BY DR. R. ROW OF BOMBAY.)

Two plant cells are shown (× 2,000).

shown that the bug *Stenocephalus agilis* is responsible for the spread of the infection from plant to plant in Portugal, while Strong (1924) has incriminated another bug (*Chariesterus cuspidatus*) in Central America. Strong, moreover, claims to have shown that lizards, which devour these bugs, acquire an intestinal infection with the flagellate, which, when

inoculated from the lizard's intestine into the skin of the monkey, produces a lesion resembling oriental sore, in which leishmania forms of the parasite occur. The evidence that the flagellate of the lizard is actually that of the bug is not quite convincing.

Phytomonas davidi (Lafont, 1909).—The flagellate has been studied most fully in Portugal by França. He has discovered the invertebrate host of the flagellate, and has described what he regards as its cycle of development. As observed in the latex, *P. davidi* has the usual leptomonas structure (Fig. 181). The body measures 16·5 to 19·5 microns in length by 1·5 in breadth. The extremities are tapering, and the flagellum measures from 10·5 to 16 microns. A peculiar feature seen in some of the organisms is a twisting or folding of the posterior portion of the flat, blade-like body of the parasite two or three times round its longitudinal axis (Fig. 184 E). That this twisting is merely the result of the medium in which the flagellate is growing is demonstrated by an observation of Shortt (1923) that if *Leptomonas ctenocephali* of the dog flea is inoculated into a small fissure made in a Euphorbia, the flagellates persist there for six days, during which some of them become longer, and show the same twisting of the posterior part of the body. The nucleus usually lies at the junction of the anterior and middle thirds of the body, with the kinetoplast about 3 microns anterior to it. Shorter flagellates are also seen, and even round leishmania forms. Multiplication is by the usual method of longitudinal division.

Culture of the flagellate was attempted by França (1914) without success, but Nieschulz (1924*d*) has successfully cultivated and maintained on blood agar a strain from *Euphorbia cereiformis* received from Franchini. He refers to the flagellate as *Herpetomonas euphorbiæ*.

Inoculation from plant to plant was attempted by Noc and Stevenel (1911), who claimed to have transmitted the infection to healthy plants by injecting material with a glass pipette. As the local inoculation seems to have produced a generalized infection in forty-eight hours, there would appear to be some doubt as to the accuracy of the result. França (1914), trying the same experiment, after over a hundred failures, only succeeded twice in producing a localized infection of the plant. As a rule, the natural infection is found only in certain parts of the plant, and it spreads gradually from twig to twig. Instead of its usual white appearance, due to the presence of starch and other granules, the latex becomes a clear liquid, in which these substances are not found. In sections of the plant, the flagellates occur in enormous numbers, sometimes as veritable emboli, in the lactiferous tubes, in which the latex has been completely changed in character. This alteration not only brings about the death of the infected part of the plant, but eventually causes degenera-

tion and abnormal growth of the parasite, apparently as a result of exhaustion of nutriment. Amongst the degenerated parasites are some forms of large dimensions. The latter may reach a length of 30 microns and a breadth of 6 microns. The kinetoplast either entirely disappears or becomes hypertrophied. This abnormal increase in size may be comparable with that of the giant forms of *Herpetomonas mirabilis* and *H. mesnili* described above, and it may be that the presence of giant forms in the fly and latex can be attributed to similar disturbances of nutrition.

The effect of the infection on the latex has been mentioned. In a section of a healthy leaf the lactiferous tubes are seen to be filled with starch and other grains, whereas in an infected leaf the tubes are com-

FIG. 182.—*Phytomonas davidi* IN A LACTIFEROUS TUBE, AS SEEN IN A SECTION OF A TWIG OF *Euphorbia segetalis*. (AFTER FRANÇA, 1914.)

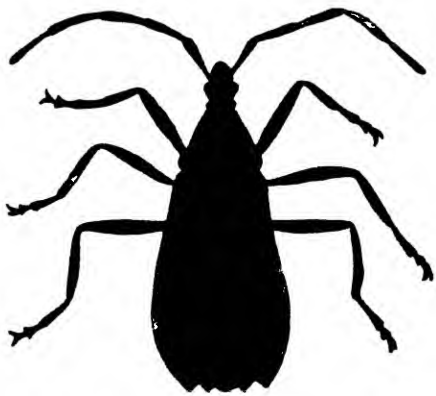


FIG. 183.—*Stenocephalus agilis* (♀), THE TRANSMITTER OF *Phytomonas davidi* IN PORTUGAL (× 3). (AFTER FRANÇA, 1920, MODIFIED.)

pletely devoid of these (Fig. 182). Furthermore, the chlorophyll gradually diminishes, and the plant finally withers and dies. Occasionally, however, an infected twig will recover.

França has also noted the flagellates in the sheath of the fruit, while in the fruit itself he has seen minute bodies which, however, he cannot certainly identify with the flagellates. He suggests the possibility of their being forms destined to infect the seeds and bring about infection of the young plants, a kind of hereditary infection analogous to the supposed infection of the ova of insects. Strong (1924) has noted that all parts of the plant, including the roots, may be infected.

A transmitting host of the flagellate has been sought by several observers. Lafont (1909) noted that the plants (*E. hypericifolia*) har-

boured hemiptera, and in one of these, *Nysius euphorbiæ*, he found a flagellate of the leptomonas type. He succeeded (1911) in infecting healthy plants by means of these bugs, but failed to infect *E. peplus*, which is never found naturally infected in Mauritius. Bouet and Roubaud (1911), employing eighty specimens of the bug *Dieuches humilis*, also succeeded in carrying infection from one plant (*E. pilulifera*) to another. Rodhain and Bequært (1911) observed flagellates in the intestine of an hemipteran larva taken off infected *Euphorbia indica* in the Congo.

França (1919 and 1920*a*), working in Portugal with *E. segetalis*, has succeeded in transmitting the infection by the agency of a bug, *Stenocephalus agilis* (Fig. 183). The bug is chiefly nocturnal in its habits, and, when feeding, punctures the leaf in many places. The points of puncture—the primary lesions—when examined, are found to contain minute rounded or slightly elongate forms of the flagellate, which are very similar to those which occur in the salivary glands of the bug. It is later that the infection extends from the primary lesion to the latex, and becomes general. França has traced the development of the flagellate in the bug up to an invasion of the salivary glands (Fig. 184). The forms ingested by the bug when feeding on infected latex multiply rapidly in the gut up to the fourth day. It is supposed that there then occurs a process of syngamy, in which two flagellates, after losing their flagella and kinetoplasts, fuse completely. Unfortunately, this appears to have been deduced from stained films only, so that it cannot be accepted as reliable. From the fourth day onwards there appear large giant forms up to 50 microns in length, and rounded multinucleate bodies. After this period, only small forms 4·5 to 7 microns are found. These are, presumably, the infective forms, for they occur, not only in the gut, but also in the salivary glands. Small round leishmania forms, some of which appeared to be encysted, were found occasionally in the hind-gut, and once in the proboscis. Invasion of the salivary glands seems to take place by a forward migration of the intestinal forms, which make their way to the proboscis and thence up the salivary duct, as in the cycle of development of *Trypanosoma gambiense* in tsetse flies. Flagellates were not found in the hæmocœle fluid, though a dipterous larva (one of the *Ocypterinæ* or *Gymnosominæ*), inhabiting the body cavity, was found infected.

Galli-Valerio (1921), working in Switzerland, has found *Euphorbia gerardiana* infected at a height of 1,300 metres above sea-level. The plants provided one specimen of *Stenocephalus*, and in this bug he claims to have found the intestinal flagellates and the small metacyclic forms in the salivary glands described by França. Franchini (1922*b*) collected the insects and bugs from a large number of infected Euphorbias near Bologna. In no case was *Stenocephalus* found, and it is concluded that other arthropods

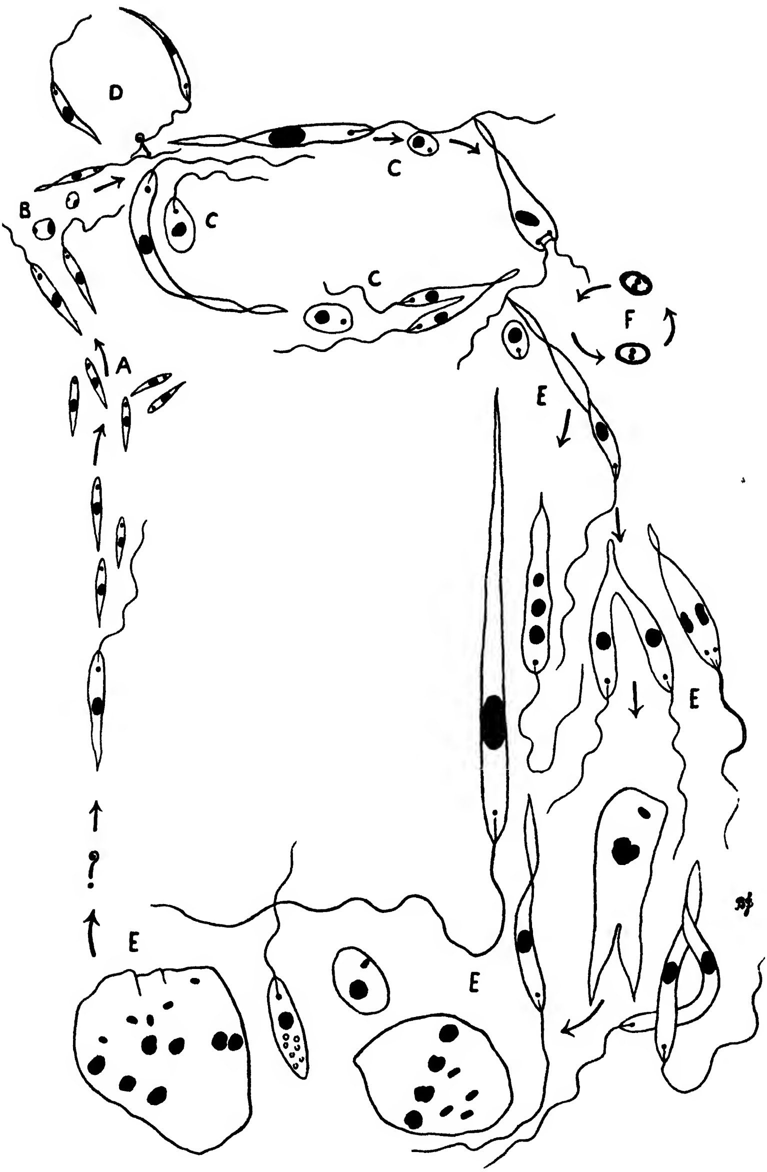


FIG. 184.—LIFE-CYCLE OF *Phytomonas davidi* AS DESCRIBED BY FRANÇA (× *ca.* 1,000). (AFTER FRANÇA, 1920.)

A. Infective forms in salivary gland of bug (*Stenocephalus agilis*).
B. Forms in primary lesion on cuticle of plant, which results from the bite of the bug.
C. Forms in latex when infection becomes generalized.
D. Forms in fruit.
E. Forms in intestine of bug.
F. Resistant forms in fæces of bug.

probably play a part in the transmission of the flagellates, a view with which França (1922) disagrees. In a later paper Franchini (1922*p*) states that he has found the flagellate in flies (*Anthomyia maculata*) taken off the plants.

Strong (1924) has published an account of experiments conducted with the flagellates of Euphorbias in Central America. He has shown that the coreid bug *Chariesterus cuspidatus* infects itself from the Euphorbias, on the juices of which it feeds. It was also noted that certain lizards (*Cnemidophorus lemniscatus*) which fed upon insects harboured in the posterior portion of the intestine a flagellate indistinguishable from that of the bugs. It was evidently of interest to investigate the connection between these flagellates and those of cutaneous leishmaniasis which occurred in the district. Monkeys, dogs, guinea-pigs and mice were inoculated intraperitoneally and subcutaneously with the flagellates from the plants, bugs, and lizards. All these experiments were entirely negative as regards the production of generalized or local infections, except in one monkey inoculated subcutaneously on the abdomen with flagellates from the lizard, in which a papule appeared on the eighth day. It increased in size, and finally ulcerated. On the sixteenth day, definite leishmania were discovered in the lesion, and these were found to be numerous in sections of the ulcer, which was removed when the animal was killed on the twenty-fourth day. As no similar lesions resulted from inoculation of the flagellates from the plants or the bugs, it is concluded that the flagellates had become capable of infecting the skin of the monkey as a result of their modification in the intestine of the lizard. As pointed out above, the proof that the flagellate of the bug is identical with that of the lizard was not obtained. In the light of the observations of França, Galli-Valerio, and Strong, it is interesting to recall the fact that several observers, as noted above (p. 370), have recorded the presence of flagellates in the salivary glands of plant bugs.

Of these plant flagellates, França (1921) recognizes three species, which are said to differ as regards the dimensions of the fully-grown leptomonas forms. He notes that the Euphorbia flagellate of Portugal may be distinct from Lafont's original form from Mauritius. Should this prove correct, he suggests the name *Leptomonas lafonti*. The dimensions in microns of the leptomonas forms of the three species of *Phytomonas*, as given by França (1921) are shown in the table below (p. 389). It must be admitted, however, that much more extensive observations will have to be made before they can be accepted as indicating specific distinctions. Fantham (1925) proposes the name *Herpetomonas ficuum* for a flagellate of *Ficus edulis*.

It has been noted above (p. 335), that França (1920*a*) believes that flagellates of the genera *Herpetomonas* and *Leptomonas* can be distin-

guished by their method of division. In the case of the former, it is claimed that the kinetoplast, rhizoplast, and entire flagellum divide; while in the latter, a new axoneme grows out from the daughter blepharoplast to form a new rhizoplast and flagellum. Nieschulz (1924*d*) apparently interprets França as making the claim that in flagellates of the genus *Leptomonas* no rhizoplast is present, though this structure is clearly shown in França's figures. Having found that in the cultural forms of the flagellate of *Euphorbia cereiformis* a rhizoplast occurs, he gives it the new name *Herpetomonas euphorbiæ*, as França groups the Euphorbia flagellates studied by him in the genus *Leptomonas*. Actually, there is no difference between the flagellate studied by Nieschulz and those studied by França.

	P. elmassiani.	*P. davidi.*	*P. bordesi.*
Length of body	12 to 15	16·5 to 19·5	24 to 27
Length of flagellum	4·5 to 7·5	10·5 to 16	7·5 to 9
Distance of kinetoplast from anterior end of body ..	1·5	1·5	2·2 to 3
Distance between the kinetoplast and nucleus	1·5	3	3
Length of nucleus	1·5	2 to 3	2·2 to 3
Distance between nucleus and posterior end of body ..	7·5 to 10·5	10·5 to 12	16·6 to 18

Laveran and Franchini (1920*a*) have discovered leptomonas in a number of Euphorbias in Italy as follows: (Bologna) *E. peplus*, *E. dulcis*, *E. falcata*, *E. nereifolia*, *E. virosa*; (Florence) *E. humifusca*; (Ferrara) *E. peplus*; (Syracuse) *E. peplus*; (Catania) *E. grandis*. In two of these, only rounded non-flagellate forms were found, and they think it possible a distinct species is represented. Euphorbias in Paris were not found infected, but an attempt was made to inoculate plants (*E. sauliana* and *E. pilosa*) with cultures of *Leptomonas ctenocephali* of fleas. As long as two months after, the inoculated twigs were not growing so well as the control ones, while smears from the latex showed typical flagellates. The examination of the controls was entirely negative. In the same paper, these observers claim to have produced a mild infection in mice by inoculating them with the flagellate of the Euphorbias. Franchini (1921*b*, 1922*c*) stated that he had found four members of the family Apocynaceæ (*Acokanthera spectabilis*, *A. venenata*, *Funtumia elastica*, and *Thevetia nereifolia*) infected, and that in *Euphorbia nereifolia* and *E. cærulescens* he had found flagellates which had the trypanosome arrangement of the kinetoplast and nucleus. These had a length up to 12 microns. The undulating membrane, when visible, was poorly developed. Rounded forms also occurred, and these appeared to be produced by the flagellate first becoming looped and the space between the limbs gradually filling up with cytoplasm. Franchini has given the name *Trypanosoma euphorbiæ* to this flagellate. Even if his statement is to be relied upon, it has yet to be demonstrated that he was not dealing with a hitherto undetected form of development of *Phytomonas davidi*.

The same observer (1922*f*) described a flagellate infection of cabbages, which were infested with various species of pentatomid bugs (*Pentatoma ornatum*, *P. ornatum* var. *pectorale*, *P. oleraceum*). These bugs commonly have an intestinal crithidia infection, and it is claimed that the flagellates sometimes invade the salivary glands.

The cabbage leaves, which are heavily infested with bugs, become yellow and unhealthy. In these, Franchini claims to have found the flagellates and leishmania forms. In a discussion which took place after the announcement, Roubaud stated that he had frequently observed the intestinal infection of the bugs, but, though he had specially looked for them, he had failed entirely to find flagellates in the salivary glands of the bugs or in the tissues of the cabbages. In another paper Franchini (1922*d*) describes as *Crithidia oxycareni* an intestinal crithidia of the bug *Oxycarenus lavateræ*, which lives in bushes of the species *Altea syriaca*. He states that he found leishmania forms of the flagellate in the fæcal deposits of the bug on the surface of the leaves, and that these forms occurred also in the tissues of the leaves. Franchini (1922*g*) states that he examined a number of latex-producing plants in the Botanical Gardens in Paris, with the following results: Flagellates of the trypanosome type were seen in five species of the family Euphorbiaceæ (*Euphorbia calyculata, E. nereifolia, E. virosa, Elæophorbia drupifera, Exocœria emmarginata*), and leishmania forms in one (*Manihot dichotoma*). Crithidia were seen in one of the Asclepiadaceæ (*Cryptosteiga grandiflora*). Of two Apocynaceæ, leptomonas were present in *Cerbera odollam*, and a large trypanosome with membrane but no flagellum in *Caudronia javanensis*. Amongst the Urticaceæ, trypanosome forms were found in *Ficus benjamina* and leishmania forms in *Ficus tholloni*. Of the Sapotaceæ examined, *Sideroxylon inerme* contained a herpetomonas (leptomonas) form, *Chrysophyllum glabrum* and *C.* sp. a large trypanosome with undulating membrane and no flagellum. The statement is made that mice were inoculated, and that trypanosomes were seen in the blood. It will be noted that in an earlier paper, Laveran and Franchini stated that the Euphorbias of Paris were not infected, and that they were successfully inoculated with *L. ctenocephali*. Franchini (1922*k*) has described the presence of flagellates of the leptomonas, crithidia, and trypanosome type in the juice of the fruit and the latex of *F. parietalis*. They have been cultivated, and with the cultures mice were inoculated. Leishmania forms were found in the blood of the animals. In a later paper (1922*m*) an account is given of attempts to infect Euphorbias with other flagellates. With cultures of *Leishmania tropica*, *E. segetalis* was infected; with *L. donovani*, *E. ipecacuanha*; and with *Herpetomonas muscarum*, *E. geniculata*. The infected plants were constantly in poor condition compared with the controls, while leishmania forms occurred regularly in the plant juices for as long as three months. Franchini (1923*a*) again claims to have successfully infected Euphorbias with the intestinal flagellates of *Musca domestica*, *Sarcophaga hæmorrhoidalis*, *Calliphora erythrocephala*, and *Pentatoma ornatum*. Shortt (1923) has noted the persistence of *Leptomonas ctenocephali* for six days after being introduced into a small excavation on a bough of a Euphorbia plant. As will be seen below, many of the statements contained in papers to which Franchini's name is attached, and which describe successful inoculations of insect flagellates to vertebrates, are of such a nature that it seems impossible to estimate their real value. It is evident that many of them cannot be accepted till reliable confirmation is forthcoming.

RECORDED PHYTOMONAS INFECTIONS OF PLANTS.

Euphorbiaceæ.

E. brasiliensis, Noguchi, 1924, Honduras.
E. callitrichoides, Strong, 1924, Central America.
E. caproni, Monti (quoted by Visentini, 1914), Sardinia.
E. cereiformis, Franchini, 1923, France.
E. cyparissias, Aubertot, 1923, Alsace. Bruni, 1925, France.
E. dulcis, Laveran and Franchini, 1920, Italy.

E. esula var. *mosana*, Zotta, 1921, France.
E. falcata, Laveran and Franchini, 1920, Italy.
E. gerardiana, Galli-Valerio, 1921 and 1923, Switzerland.
E. grandidens, Franchini, 1923, Italy.
E. grandis, Laveran and Franchini, 1920, Italy.
E. helioscopia, Franchini, 1923, France; Aubertot, 1923, Alsace.
E. humifusca, Laveran and Franchini, 1920, Italy.
E. hypericifolia, Lafont, 1909, Mauritius; Vincent, 1910, Reunion; Noc and Stevenel, 1911, Martinique and Antilles; Iturbe, 1918, Venezuela; Strong, 1924, Central America.
E. indica, Rodhain and Bequært, 1911, Belgian Congo.
E. nereifolia, Laveran and Franchini, 1920, Italy; Franchini, 1923, Italy.
E. neruri, Row, 1915, Bombay.
E. officinarum, Franchini, 1923, Italy.
E. peploides, Sergent, Et., 1921, Algeria.
E. peplus, França, 1911, Portugal; Laveran and Franchini, 1920, Italy.
E. pilulifera, Lafont, 1909, Mauritius, and 1911, Madagascar, Mayotte, and Zanzibar; Donovan, 1909, Madras; Vincent, 1910, Reunion; Carougeau and le Fera, 1910, Madagascar; Bouet and Roubaud, 1911, Dahomey; Leger, 1911, Upper Senegal and Niger; Noc and Stevenel, 1911, Martinique; Lebœuf and Javelly (v. Laveran and Mesnil, 1912, França, 1914), New Caledonia; Row, 1915, Bombay; Tejera, 1919, Venezuela; Strong, 1924, Central America; Noguchi, 1924, Honduras.
E. schimperiana, Monti (quoted by Visentini, 1914), Sardinia.
E. secretalis (*segetalis ?*), Tejera, 1919, Venezuela.
E. segetalis, França, 1911, Portugal; Visentini, 1914, Italy.
E. splendens, Franchini, 1923, Italy.
E. striata, Fantham, 1925, S. Africa.
E. thymifolia, Lafont, 1909, Mauritius, and 1911, Madagascar and Mayotte; Vincent, 1910, Reunion; Carougeau and le Fera, 1910, Madagascar; Row, 1915, Bombay; Tejera, 1919, Venezuela.
E. virosa, Laveran and Franchini, 1920, Italy; Franchini, 1923, Italy.

Asclepiadaceæ.

Araujia angustifolia, Migone, 1916, Paraguay; Cordero (quoted by França, 1921), Uruguay; Franchini, 1923.
Cynachum acutum, Zotta, 1923, Roumania.
Morreira odorata, Migone, 1921, Paraguay.
Asclepias curassavica, Hegner, 1924, and Noguchi, 1924, Honduras.
Asclepias syriaca, Holmes, 1924, Baltimore; Noguchi, 1924, New York.

Apocynaceæ.

Acokanthera spectabilis, Franchini, 1922, Italy.
Acokanthera venenata, Franchini, 1922, Italy.
Cerbera odollam, Franchini, 1922, Paris.
Funtumia elastica, Franchini, 1922, Italy.
Thevetia nereifolia, Franchini, 1922, Italy.

Sapotaceæ.

Sideroxylon inerme, Franchini, 1922, Paris.

Urticaceæ.

Ficus parietalis, Franchini, 1922, France.
Ficus benjamina, Franchini, 1923, Italy.
Ficus edulis, Fantham, 1925, S. Africa.

INOCULATION OF INSECT TRYPANOSOMIDÆ INTO VERTEBRATES.

A number of investigators, particularly Laveran and Franchini, and Fantham and Porter, have claimed that vertebrates, particularly mice, may be infected easily with insect flagellates by inoculation or feeding. In some cases it is stated that a definite disease condition resembling kala azar has resulted, and that the infection can be handed on from animal to animal by inoculating emulsions of the infected organs. The infection is associated with the presence of leishmania forms in smears of the organs, while sometimes actual leptomonas forms occur in the blood. The experiments of these investigators have been repeated by a number of competent observers, who have failed entirely to substantiate their claims. It would seem probable that some fallacy, such as the interpretation as leishmania of structures which are of another nature, has been responsible for the very high percentage of positive results claimed. The only reliable test of an infection is the discovery of undoubted parasites in smears of the blood or organs, or the development of flagellates in cultures made from the blood or organs on N.N.N. or other suitable medium.

After Basile's claim that Mediterranean kala azar was transmitted from dog to man by the dog and human fleas, *Ctenocephalus canis* and *Pulex irritans*, had become known, the relation of the naturally occurring flea flagellates to *Leishmania donovani* became the subject of many investigations. The question was raised as to whether the insect flagellates could give rise to infections when inoculated into vertebrates. Laveran and Franchini (1913) published an account of the infection of mice with *Leptomonas ctenocephali*. After inoculation by the intraperitoneal route, the parasites were found by direct examination in the peritoneal exudate and in the blood for as long as sixty days. In the blood, both leishmania and leptomonas forms occurred, while after death leishmania forms were found in the smears of liver and spleen. Mice inoculated with peritoneal exudate of inoculated mice also acquired an infection. Later (1914*a*, 1919, 1920) successful infections of mice, rats, guinea-pigs, dogs, and monkeys (*Macacus cynomolgus*) were reported. Mice were readily infected by inoculation of emulsions of the organs of infected mice, while rats were infected by inoculation with heart blood, and dogs with spleen emulsion of infected mice. Again, in other papers (1914*b*, 1914*c*, 1919*a*) it is recorded that, working with *L. pattoni* of *Ceratophyllus fasciatus*, rats and mice were found to be susceptible to inoculation and feeding. Rats and mice placed in jars with infected fleas for forty-eight hours became infected with *L. pattoni*, and it was shown that mice could be infected by contaminating their food with infected fleas. Mice were also infected by the oral administration of *Crithidia melophagia* (*Trypanosoma melophagium*). Experiments (1913*a*, 1914*a*) were also carried out with rats and mice and *Crithidia fasciculata* of *Anopheles maculipennis* with similar results. This flagellate was also inoculated from one mouse to another, and an interesting result was obtained by cutaneous injection. A local sore developed, in which leishmania forms were said to occur. There was also a general infection at the same time. Galli-Valerio (1923) also states that more than two months after inoculation of a rat with the flagellates from *Melophagus ovinus* the animal died, and leishmania forms were found in its organs.

Franchini and Mantovani (1915) also claim to have infected rats with *Herpetomonas muscarum* of house flies. They state that they obtained a culture from the heart blood of an inoculated rat in N.N.N. medium. The only organisms seen in the cultures had the appearance of anaplasma, and they claim that mice were successfully inoculated by means of the cultures. The mice showed leishmania forms in their organs. It is impossible to understand what the authors mean by the small anaplasma forms, which were apparently the only ones seen in the culture. It is difficult to conceive of a culture of *H. muscarum* which would not show the usual leptomonas forms. Laveran and Franchini (1919*a*, 1920) report that mice and guinea-pigs were infected by inoculation of cultures of *L. ctenocephali*, which was again recovered by culture from the blood. Similarly (1919*a*), cultures of *L. jaculum* of the water bug *Nepa cinerea* were obtained by inoculating mice intraperitoneally with the intestinal contents of the bugs and cultivating from the heart blood, and mice were infected by inoculation of cultures of *C. melophagia*. The infections were carried on to other mice by injections of liver and spleen material. Laveran and Franchini (1920, 1920*b*) gave accounts of successful experiments with cultures of the leptomonas of *Phlebotomus*. Two dogs were inoculated in the skin of the thigh. One developed a local lesion in which large cells containing numerous leishmania occurred, while the other acquired a general infection (see p. 436). Guinea-pigs and mice were also infected, and leishmania and other forms found in the organs. Roubaud and Franchini (1922) state that several mice, which were placed in jars in which fleas (*Ctenopsylla musculi*) were breeding, acquired infections, and that leishmania forms in which the kinetoplast was not clear were found in the organs. From the spleen of one of these mice another mouse was infected. They also claim (1922*a*) that mice inoculated subcutaneously with dried fæces of fleas became infected. In a later paper these authors (1923) state that a culture was made from the heart blood of one of the mice two and a half months after its inoculation. Nothing appeared in the culture for some time, but over three months later the tube, which had been put aside, was examined and flagellates were found. Subcultures were successfully obtained. It is evident that if flagellates took such a long time to appear in the cultures, they must have been exceedingly scanty in the heart blood of the mouse. Laveran and Franchini (1923) give an account of experiments conducted with the flagellates of the bug *Pentatoma ornatum*. These were inoculated to mice and passed through other mice in series. In all cases infection resulted, though it is admitted that the organisms were present in small numbers only. These were said to be of the leishmania, piroplasma, or anaplasma type. Cultures were repeatedly made from the heart blood or organs of the experimental animals, but in only one case was a positive result obtained. In this culture only round forms were present, no flagellates being seen. The figures accompanying the description serve a useful purpose in that they illustrate what the author is willing to accept as evidence of infection in animals.

The organisms discovered in infected animals by Franchini and those who have associated themselves with him were usually of the leishmania type, though the elongated flagellates were often said to be present in the blood-stream and occasionally in the organs. As a rule, the parasites were scanty in number, the animals not showing the intense infection which sometimes occurs in mice inoculated with *Leishmania tropica* or *L. donovani*. Some of the figures, or rather diagrams, produced by these observers, however, show large cells of the macrophage type packed with parasites, as seen in oriental sore and kala azar. Experiments of a similar kind have been recorded by Fantham and Porter (1915*a*). Working with *L. jaculum* of the water bug *Nepa cinerea*, they claim to have successfully infected mice by inoculation or feeding with the intestinal contents of the bugs. A puppy, like-

wise, is described as becoming infected after being made to ingest fleas, some of which harboured *L. ctenocephali.* A more extensive series of experiments was published later (1915*b*). In these, four flagellates were used (*L. jaculum, L. stratiomyiæ, L. pediculi,* and *C. gerridis*), and various vertebrates as follows: the stickleback (*Gasterosteus aculeatus*), newt (*Molge vulgaris*), frog (*Rana temporaria*), toad (*Bufo vulgaris*), lizard (*Lacerta vivipara*), snake (*Tropidonotus natrix*), and mice (*Mus musculus*). These animals were infected with one or more of the flagellates, either by inoculation or feeding. In many cases, Fantham and Porter believe that the organisms acted as pathogenic agents, and brought about the death of the animals. Still another series of experiments is recorded by these observers (1915*c*). On this occasion, they claim to have infected birds (canaries, martins, sparrows) by feeding them with *L. jaculum* or *L. culicis* of *Culex pipiens.* In these experiments they claim to have found leishmania and flagellate forms of the parasites in the blood and various organs, and state that the birds became ill from the infections induced. It is suggested that it is possible that in nature these infections may be one of the causes of mortality amongst birds.

The remarkable feature of all these experiments is the apparent ease with which infections were produced. Other workers, as, for instance, Nöller (1912*d*), failed to infect a young dog with *L. ctenocephali.* Chatton (1919) failed entirely to infect mice with the same flagellate, and the writer has had a similar experience with the cultures of the leptomonas of *Pulex irritans.* Tyzzer and Walker (1919) conducted very careful experiments with *L. ctenocephali.* Though they inoculated mice, some of which were newly-born, by various routes, they never succeeded in producing an infection. Patton (1921) has stated that he has failed entirely to infect mice with several species of insect flagellate, while Glaser (1922) attempted without success to repeat Franchini and Mantovani's experiments with *H. muscarum.* Hoare (1921*a*) made a very careful study of the question, and carried out a series of experiments with the flagellates of *Calliphora* sp., *Nepa cinerea,* and *Melophagus ovinus,* The vertebrates inoculated or fed with one or other of these flagellates were mice. newts, frogs, and sticklebacks. Though very searching observations were made, involving not only the examination of smears, but also cultures from the heart blood and organs, in no single instance was an infection demonstrated. Hoare's experiments indicate, at any rate, that infections cannot easily be produced, and that the claim that purely insect flagellates may take on pathogenic properties seems very doubtful indeed. As Hoare points out, in conducting experiments of this kind, only undoubted leishmania forms should be accepted as evidence of infection. In the successful experiments recorded above, the observers have undoubtedly been willing to accept as leishmania forms bodies of a doubtful nature. This is clearly shown by the frequent references to leishmania forms with a single nucleus and the anaplasma forms in cultures. Roubaud and Franchini (1922), for instance, state that the parasites in the infected mice mostly had a single nucleus, and that the kinetoplast was very difficult to distinguish. In the absence of a kinetoplast, it is not easy to comprehend the reasons for regarding the bodies as flagellates at all. They might equally well be yeasts, the organism which has been named *Encephalitozoon,* or the merozoites of some Sporozoon such as *Klosiella,* which may infect the endothelial cells of the bloodvessels.

Glaser (1922) made unsuccessful attempts to infect six mice, a rat, and a guinea-pig with *H. muscarum,* while Shortt (1923*a*) conducted a series of experiments with *L. ctenocephali* of the dog flea, and *L. luciliæ* of *Lucilia craggii,* and rats, mice, monkeys, dogs, pigeons, and frogs. The animals were either fed or inoculated in various ways, and were subsequently examined by the smear and culture method. Over fifty experiments were made, and in not a single instance was

PLATE III.

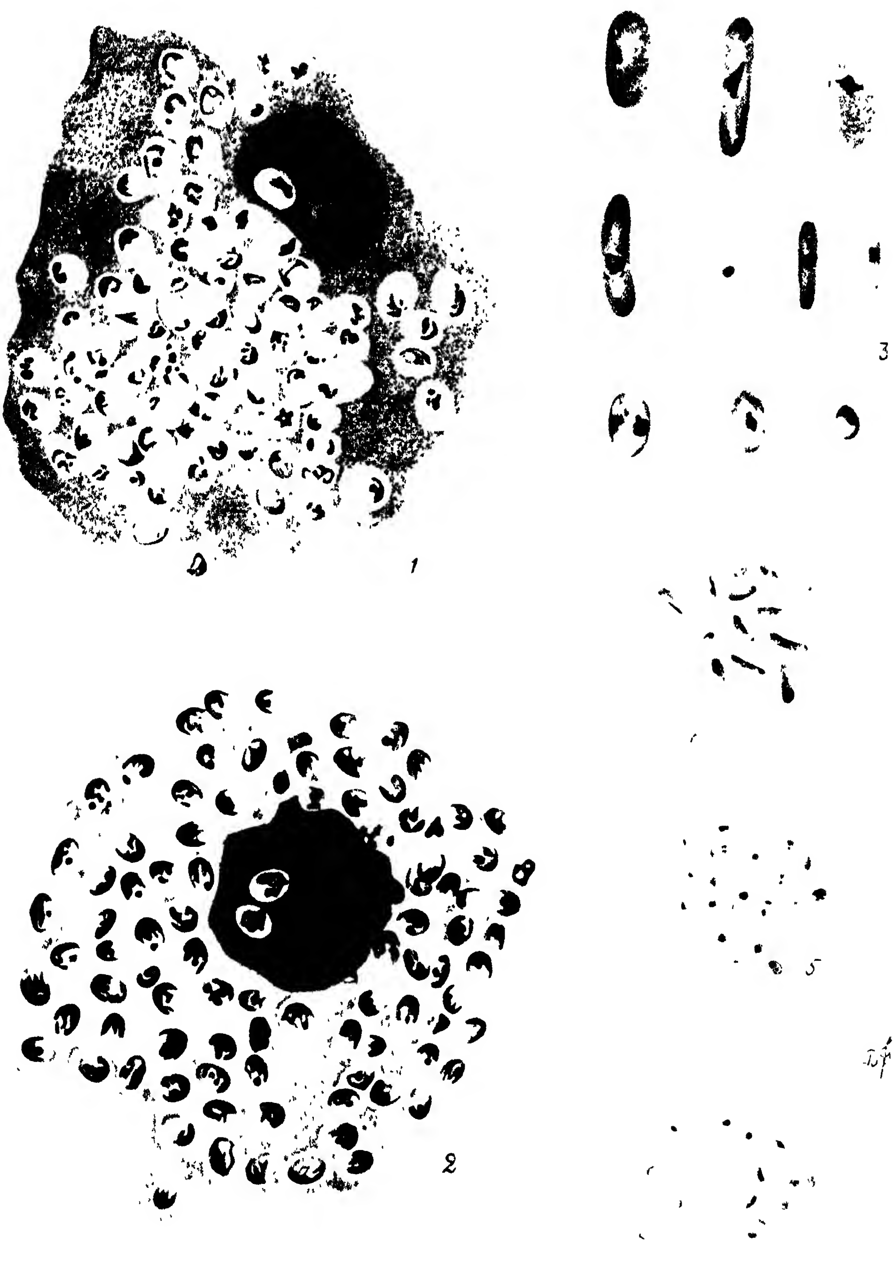
VARIOUS VEGETABLE ORGANISMS WHICH SIMULATE PROTOZOA WHEN THEY OCCUR IN DRIED BLOOD-FILMS OR SMEARS OF ORGANS STAINED WITH ROMANOWSKY STAINS. (1 and 2, ×2000: 3-6, ×1000)

1. *Histoplasma capsulatum* in macrophage from smear of human lymphatic gland. Note resemblance to *Leishmania.*
2. *Cryptococcus farcinimosus*, the cause of lymphangitis of horses, from smear of lymphatic gland. Note resemblance to *Leishmania.*
3. Group of large vegetable cells in a blood-film contaminated with intestinal contents of a rabbit. They bear some resemblance to hæmogregarines.

4-6. Groups of yeast-like organisms in blood-films contaminated from cultures. They may be confused with *Leishmania*, merozoites of Sporozoa, or spores of Microsporidia.

(1 AND 2, AFTER ROCHA-LIMA; 3-6, ORIGINAL.)

PLATE III.



an infection noted. Yamasaki (1924) also failed to infect mice and dogs with *L. ctenocephali*.

Becker (1923*a*), using *C. gerridis*, *H. muscarum*, and the flagellates of the sheep ked, failed entirely to produce infection in seventeen rats, three mice, one rabbit, and one guinea-pig. As in the case of Hoare's and Shortt's experiments, cultures on N.N.N. medium were attempted. Strong (1924) likewise failed to infect animals with the flagellates of the bugs found on Euphorbias, while Drbohlav (1925) failed to produce any infection with *L. ctenocephali* in about 150 animals examined by the smear and culture method after inoculation.

There is no longer any doubt that the *C. melophagia* of the sheep ked is in reality *T. melophagium* of the sheep, yet, by inoculation of this trypanosome to mice, Laveran and Franchini claim to have obtained infections of the leishmania type. As pointed out by Hoare (1921*a*), if an infection had occurred, it would almost certainly have been of the ordinary trypanosome type. Buchner (1922), who also failed to infect mice with the ked flagellate, likewise points out this fallacy in the experiments of Laveran and Franchini. It seems impossible to accept the remarkable statements regarding the successful inoculation of insect flagellates to vertebrates till definite confirmation is forthcoming. The negative results obtained by so many competent observers suggest that the positive results reported may have been due to misinterpretations. It is possible, however, that occasionally isolated parasites may survive in the organs and give rise to cultures, but it is doubtful if such a condition can be regarded as an infection. There is certainly no reliable evidence that, even if such a survival of the parasites occurs, they give rise to serious and fatal disease. Though Fantham and Porter claimed that sticklebacks were killed by the infections induced by feeding them on the intestines of the water bug, Hoare found that the fish thrived on this diet.

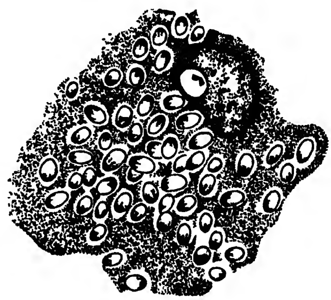


FIG. 185.—*Cryptoroccus muris* (× *ca.* 1,000). (AFTER SANGIORGI, 1922.)

There occur in the organs of rats and mice structures which can readily be mistaken for leishmania. Thus, Sangiorgi (1913) has described as *Toxoplasma musculi* certain minute bodies found by him in the spleen of a mouse, and as *T. ratti* similar forms from the rats. Sangiorgi (1922*b*) also recorded a *Cryptococcus* from mice, and it is this organism which was named *C. muris* by Shortt (1923*a*), who discovered it in mice in India (Fig. 185). Whether the structures described by Sangiorgi are Toxoplasmata or not, it is evident that they and the cryptococcus could be easily mistaken for leishmania. The same remarks apply to the parasite of rabbits described as *Encephalitozoon cuniculi* and the similar form in mice, both of which occur fairly commonly in the organs of laboratory animals (p. 754).

INOCULATION OF INSECT TRYPANOSOMIDÆ INTO INVERTEBRATES.

As trypanosomes can be inoculated from one vertebrate to another, so can invertebrates be inoculated with flagellates obtained from other invertebrates. Zotta (1912) observed a leptomonas in *Pyrrhocoris aptera*, a plant bug. The infection occurred, not only in the intestine, but also

in the body cavity, whence all the organs of the body were invaded. He (1921) succeeded in obtaining a culture of the organism *L. pyrrhocoris* in N.N.N. medium. In the same year (1921*a*) he investigated the effect of these cultures on other arthropods by inoculating them in the body cavity. He found that active multiplication occurred, some of the experimental arthropods becoming overrun with flagellates. In this manner he succeeded in infecting *Notonecta glauca* (water boatman), *Naucoris cunicoides* (aquatic bug), *Galleria mellonella* (caterpillar of bee-hive moth), *Calliphora* sp. (larva of blow-fly), *Tenebrio molitor* (larva of meal-worm). The most intense infections were produced in the larva of the meal-worm and the caterpillar. Glaser (1922) has similarly succeeded in infecting *Melanoplus femurrubrum* (grasshopper) and *Amblycorypha oblongifolia* (locust) with *H. muscarum* of the house fly.

By feeding bed bugs on cultures of *Leptomonas pulicis*, *Crithidia ctenocephali*, and *Herpetomonas muscarum*, Patton, La Frenais, and Rao (1921) have shown, by making cultures from the intestine in N.N.N. medium at varying intervals after feeding, that the flagellates can survive for thirty-seven, eight, and forty-five days respectively.

Genus: Leishmania Ross, 1903.

The flagellates included in this genus are characterized by the possession of both a vertebrate and an invertebrate host, as in members of the genus *Trypanosoma*, from which they differ in that only leishmania and leptomonas forms occur in the cycle of development. In no case, however, has an invertebrate host actually been demonstrated, but the evidence that such a host exists is so convincing that this feature has been included in a definition of the genus.

From the purely morphological point of view there are at present no data which afford a means of distinguishing members of the genus *Leishmania* from those of the genus *Leptomonas*. In both there occur only the leishmania and leptomonas forms. The members of the genus *Leptomonas* are handed on from one invertebrate to another by the contaminative method by means of encysted forms passed in the fæces. No such stages are known in the case of *Leishmania*, though they may occur. It would thus be quite logical to include *Leishmania* in the genus *Leptomonas*. Nevertheless, on account of the existence of two hosts in the former and a single one in the latter, the retention of the separate genera is a convenience. The inclusion of *Leishmania* in the genus *Herpetomonas*, as Patton and others have done, cannot be admitted, as the members of the genus *Herpetomonas* have definite trypanosome stages which do not occur in *Leishmania*.

The first observer to see one of the parasites which are now regarded

as belonging to the genus *Leishmania* was Cunningham (1885) in India, who described " Peculiar Parasitic Organisms in the Tissue of a Specimen of Delhi Boil." The parasitic organisms referred to were the large macrophages which were supposed to be amœbæ, while the leishmania within them were regarded as spores. Firth (1891), who made similar observations, proposed the name *Sporozoa furunculosa* for the large cells containing the spores. As the name was given primarily to the supposed amœboid forms which are now known to be tissue cells, Firth's name cannot be employed for the parasites.

Leishmania were next seen and recorded by Marchand (1904) in the spleen of a Chinaman who had died in Germany. A demonstration was given before the Leipzig Medical Society on February 3, 1903. This observer inclined to the view that the bodies within the large cells were degeneration products of nuclei. On May 30, 1903, appeared Leishman's paper on " The Possibility of the Occurrence of Trypanosomiasis in India," wherein he described the parasites which he had found three years before in cases of dum-dum fever. He recognized their resemblance to the round forms which occurred in trypanosome infections. On July 11 of the same year Donovan (1903) recorded the presence of the same parasites in this disease. Laveran and Mesnil (1903, 1903*a*) examined some of Donovan's films, and, owing to the scarcity of the parasites and the fact that many appeared adherent to red blood-corpuscles, they regarded them as piroplasmata, and proposed the name *Piroplasma donovani* (November 3, 1903). Ross came to the conclusion that the organism was a Sporozoon, and suggested the name *Leishmania* (November 14 and 28, 1903). The name for the organism of kala azar is, therefore, *Leishmania donovani* (Laveran and Mesnil, 1903). In March, 1904, appeared Bentley's announcement of the discovery of the organism in cases of kala azar. Nicolle (1908) gave the name *Leishmania infantum* to the parasite causing kala azar in the Mediterranean area.

Wright (December, 1903) described a similar organism from a case of oriental sore in an Armenian child who had been brought to Boston. He proposed the name *Helcosoma tropicum* for the parasite, which he considered to be a Protozoon allied to the Microsporidia. Marzinowsky and Bogroff (1904) in Russia discovered the organism in a sore on a boy who had resided in Persia, and proposed the name *Ovoplasma orientale*. Subsequent investigations have shown that the organism is morphologically indistinguishable from that of kala azar, and must be included in the same genus. The correct name for the parasite of oriental sore is *Leishmania tropica* (Wright, 1903). Rogers (1904) made the important discovery that flagellates developed in sodium citrate solution to which spleen pulp containing *L. donovani* had been added. At first he regarded them as

trypanosomes, but later came to the conclusion that they were herpetomonas (leptomonas) developed by growth of the leishmania. Leishman's original view as to the flagellate nature of the bodies was thus fully established. It was not till four years later that Nicolle (1908*b*) and Nicolle and Sicre (1908) obtained a similar culture from the leishmania of oriental sore, an observation which demonstrated more clearly the close relationship of the two parasites. Subsequent work established the fact that the parasites were the actual causes of the two diseases, which were shown to have a wide distribution in the Old World, while cutaneous leishmaniasis was found to occur also in South and Central America, where Vianna (1911) gave the name *L. brasiliensis* to the parasite. It was further demonstrated that dogs are liable to the same two diseases.

The organisms belonging to the genus *Leishmania*, which infect human beings, are thus to be regarded as flagellates of the leptomonas type, which in man and the dog are almost invariably in the leishmania stage, though very rarely the leptomonas form has been observed. There occur, however, certain flagellate infections of other vertebrates in which the predominating forms are of the leptomonas type. These organisms also will be considered as belonging to the genus *Leishmania*. Dutton and Todd (1903) stated they had seen a flagellate of the leptomonas type in Gambian house mice, but a later examination of stained films led Todd (1914) to the view that the flagellate was really a trypanosome (*T. acomys*). Balfour (1916) called attention to the fact that he and Archibald some years earlier had seen such a flagellate in the gerbil in the Sudan, but in neither of these cases was the structure of the organism accurately determined.

The Sergents, Ed. and Et. (1907), observed flagellates of the leptomonas type in a stained blood-film of a pigeon in Algiers. The body of the organism was 17 to 20 microns in length, while the flagellum measured 19 to 35 microns. The figures show an organism very similar to *Herpetomonas muscarum*. It was only found in a film made on one occasion, and has never been rediscovered. Knuth (1909*a*) found similar forms in smears of the heart blood of a roebuck in Africa, but the animal had been dead some time, was partly devoured and decomposed, and was infested with fly larvæ, so that the origin of the flagellates was doubtful. They may have been deposited by flies. Fantham and Porter (1915) gave a figure and description of a similar form observed by them in the living condition in a mouse in England. As the flagellate was seen only in the fresh blood, and was described as very active, it is difficult to understand their statement that the drawings were made with the camera lucida. A nucleus and kinetoplast, which are exceedingly difficult to detect without staining, are clearly shown.

The Sergents, Lemaire, and Senevet (1915) demonstrated the presence

of flagellates of the leptomonas type in the North African gecko (*Tarentola mauritanica*) by making cultures from the heart blood. Bayon (1915) discovered flagellates of this type in the cloaca of the chameleon (*Chamæleon pumilus*) of Robben Island, an observation confirmed by the writer (1921) for *Chamæleon vulgaris* of Egypt. Another form was found by Leger, M. (1918*b*), in the blood of a lizard (*Anolis* sp.) of Martinique. Fantham and Porter (1920) have described and figured a leptomonas from the blood of a South African fish (*Dentex argyrozona*), while Laveran and Franchini (1921), under the name of *Herpetomonas myoxi*, record a similar form from the dormouse (*Myoxus glis*) of Italy (see p. 442). Strong (1924) has seen flagellates of the leptomonas type in the intestine of the lizard (*Cnemidophorus lemniscatus*) of Central America.

In the case of the flagellates which are only seen in the blood in the living condition, it is always possible that they were in reality trypanosomes or crithidia stages of these. This possibly applies to the forms seen by Balfour in the gerbil, by Fantham and Porter in the mouse, and by Laveran and Franchini in the dormouse. Quite recently the writer saw very active flagellates in the urine of a rat. At first they were thought to be leptomonas, but more careful study of the shape and movements produced the impression that they were crithidia. Stained films, however, proved that only trypanosomes of the *T. lewisi* type were present, and as the rat was infected with this trypanosome, it was evident the trypanosomes had passed into the urine from a wound made at the autopsy.

Richardson (1925, 1926) found numerous leishmania in the spleen of a horse which died in Uganda. The writer saw the films, which resembled those from cases of kala azar. Curson (1926) has given the name *Leishmania capræ* to supposed leishmania seen in films made from the ear of a goat in S. Africa.

As regards the various species of *Leishmania* described from man, it is generally admitted that they are morphologically indistinguishable from one another. Little assistance has been obtained from animal inoculations, for it has been found that *L. donovani*, which produces a generalized infection in man, may give rise to purely cutaneous lesions in animals, as also occasionally in man; while *L. tropica*, which causes local cutaneous lesions in man, may produce generalized infections in animals. Attempts have been made to differentiate the species by serological tests, the use of which for the separation of true species is of very doubtful value. The most precise statements are those of Noguchi (1924). He employed strains of *L. donovani*, *L. infantum*, *L. tropica*, and *L. brasiliensis*. Rabbits were inoculated intravenously on four occasions at five to seven day intervals. The sera from these animals were then used on cultures to test their agglutinating power. It was found that in dilutions of $\frac{1}{10}$, or

even $\frac{1}{100}$, the serum of the animals inoculated with *L. donovani* agglutinated this organism and *L. infantum*, but not the two others. Similarly, the serum from an animal inoculated with *L. tropica* agglutinated this organism alone, and the same was true of the serum of an animal inoculated with *L. brasiliensis*. From these reactions it appears that serologically the organisms tested fall into three groups, in conformity with the clinical types of disease produced. If the sera were added to the culture media, they were similarly specific in changing the character of the growth of the homologous organisms.

LEISHMANIA IN MAN.

The Parasite of Kala Azar.

Leishmania donovani (Laveran and Mesnil, 1903).—This organism, which is often referred to as the Leishman-Donovan body, is a rounded, non-flagellate stage of a flagellate which infects the vascular endothelium and wandering macrophages of human beings, and produces the disease known as kala azar.

DISTRIBUTION.—Kala azar occurs in India, in Madras and in the district north of the Bay of Bengal, in Calcutta and along the Ganges and Brahmaputra, in Bengal and Assam. Cunningham and Pundit (1925) have recently discovered the disease in the extreme South of India opposite Ceylon. In China it occurs north of the Yang-tse in a district between the coast and a line joining Pekin and Hankow. It has also been recorded from Sumatra by Smits (1916), but from information the writer has received there appears to be considerable doubt regarding this observation. In Southern Russia it is found both west and east of the Caspian Sea, in Transcaucasia and Turkestan, while Külz (1916) has found it to be endemic in Mesopotamia. The whole of the Mediterranean littoral and many of the islands are homes of the disease, as also an area on the Blue Nile west of Abyssinia extending as far as Khartoum in the north and towards Kodok in the south. The writer has received information that the disease has been discovered in Kenya Colony.

A case in a child has been recorded by Bouilliez (1916) near Lake Chad, and another by Tournier (1920) in the Gaboon, both in West Africa.

SYMPTOMOLOGY.—The disease occurs most usually in children or young adults, and is due to invasion of the endothelial cells of the capillaries by the parasites, which are mostly concentrated in the spleen, bone marrow, and liver, though the lymphatic glands, or, indeed, any organ, may be found infected. The symptoms produced are chiefly enlargement of the spleen and liver, progressive emaciation, anæmia, and an irregular type of fever. Other symptoms may occur, such as enlargement of the

lymphatic glands, pigmentation and dryness of the skin, and œdema. These may be ascribed to the general malnutrition, while dysentery, cancrum oris, and pneumonia are complications due to secondary bacterial infections. Left untreated, the disease nearly always ends fatally, though a small percentage of recoveries may take place. Recovery has also been noted after certain bacterial infections, which seem to act adversely on the leishmania. The duration of untreated cases may be only a few months in acute forms of the disease, or several years in the more chronic type.

RELATION OF INDIAN KALA AZAR TO THE SIMILAR DISEASE IN OTHER LOCALITIES.—Kala azar was first recognized as a distinct disease in India, but after the discovery of the characteristic leishmania as its cause, it was soon found to have a much wider distribution. The discovery of kala azar in the Mediterranean area as a disease which affected chiefly very young children at once raised the question of its identity with that of India. The parasite causing the Mediterranean type of the disease known as infantile kala azar was named *Leishmania infantum* by Nicolle (1908). The discovery of the disease in the Caspian region and in the Sudan, where children and young adults are mostly affected, and the realization that in India it is by no means limited to adults, as at one time was supposed, have raised doubts as to the validity of the species *L. infantum.*

Morphologically, there is no distinction between the leishmania from the various areas in which the disease occurs, nor is there any marked difference in which animals respond to inoculation. In the Mediterranean and Caspian areas, the disease is associated with a similar one in dogs, whereas in India kala azar in dogs has not been discovered, though it has been very carefully looked for. It is known, however, that dogs can be infected with the Indian parasite. There seems no reason, therefore, to separate the Mediterranean leishmania from that of India, and the parasite of kala azar, wherever it occurs, will be designated *Leishmania donovani*. Noguchi (1924) has found that serologically *L. donovani* and *L. infantum* are identical.

PATHOLOGY.—The chief histological change in the organs of kala azar cases is an increase in the large macrophages, which are presumably derived from the endothelial cells of the capillaries. Correlated with this is an increase in the proportion of mononuclears in the blood, though the general leucocyte picture is usually one of leucopenia. The very much enlarged spleen shows an increase in fibrous tissue, and a multiplication of the macrophages, which are often loaded with parasites (Fig. 186). Similar changes occur in the liver, where the fibrotic change may be very marked, while the bone marrow shows a great increase in these large cells

(Fig. 187). In whatever part of the body parasites are found—and they may occur in any organ or tissue—they are practically always within the cytoplasm of large cells of the endothelial type. It was Christophers (1904) who first showed that, pathologically, kala azar was essentially an infection of the endothelial cells of the blood-vessels. It must be remembered that in smears of organs or in blood-films, the parasites are often seen extracellularly, but, though such forms must occur in the passage of parasites from cell to cell, the extracellular position as usually seen is due to the breaking-up of the large cells in preparation of the films.

FIG. 186.—SECTION OF HUMAN SPLEEN (× 750): *Leishmania donovani* WITHIN MACROPHAGES. (AFTER NATTAN-LARRIER, 1913.)

Not infrequently, portions of the cytoplasm, fragmentation bodies, of these large cells are broken off in the process of film-making, and if found to harbour parasites, they may produce an appearance of multiple segmentation, especially when the outlines of the organisms are imperfectly stained. In sections of tissues where artificial rupture of the cells has not taken place, the parasites are practically always found to be intracellular. Furthermore, in films, parasites are sometimes seen lying over red blood corpuscles, and have been described as actually within these cells, like the malaria parasite and piroplasmata. This is merely an

appearance artificially produced in preparation. The organism may be found in films of the peripheral blood, either in cells of the mononuclear or polynuclear variety.

The parasites, as already remarked, may occur in any tissue of the body within the macrophages. They were demonstrated by Christophers (1904) in intestinal ulcers, and in the papules which sometimes occur in the skin of cases of kala azar. Bramachari (1922) has noted that cases

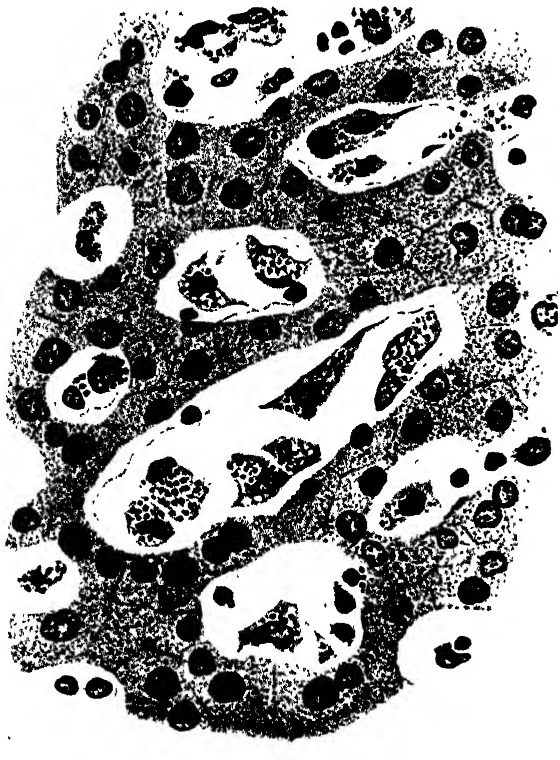


FIG. 187.—SECTION OF HUMAN LIVER (× 750): *Leishmania donovani* WITHIN MACROPHAGES AND THE GLANDULAR CELLS. (AFTER NATTAN-LARRIER, 1913.)

of the disease which have apparently recovered after antimony treatment may develop nodules on the skin, which in one case were distributed over the body, and resembled a form of nodular leprosy. Leishmania were present in all these lesions, though they had apparently disappeared from the internal organs. Another similar case has been described by Shortt and Bramachari (1925). Perry (1922) has found that in cases of kala azar the subepithelial tissues of the wall of the intestine may be much swollen, owing to the presence of enormous numbers of macrophages packed with

leishmania (Fig. 188). This condition has led him to suggest the possibility of the spread of infection by the escape of parasites from the body in the dejecta. Shortt (1923*c*), and Shortt, Swaminath, and Sen (1923), have demonstrated the escape of *L. donovani* in the urine.

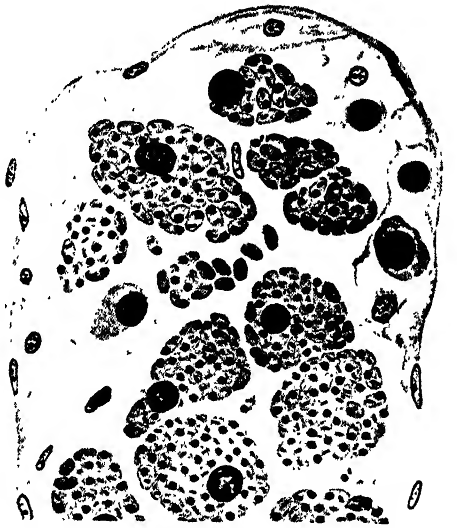


FIG. 188.—SECTION OF DISORGANIZED VILLUS OF SMALL INTESTINE, WITH *Leishmania donovani* IN MACROPHAGES (× *ca.* 750). (AFTER PERRY, 1922.)

DIAGNOSIS BY DISCOVERY OF THE PARASITE.—Diagnosis of the disease is established by the discovery of the parasite. This is usually accomplished by making films of material obtained by puncture of the spleen, and staining by Romanowsky stain. The operation is not entirely free from danger, as in some cases, especially when the spleen is very soft and large, the wound has continued to bleed and death has resulted. When due care has been taken, however, there is little risk of hæmorrhage. An ordinary hypodermic syringe may be employed, and it should be perfectly dry. The best result is obtained when very little blood is abstracted, on which account suction should be discontinued as soon as blood appears above the needle. If this be done, there will be a greater number of spleen cells, which are the ones required for finding the parasites. The less dangerous operation of liver puncture will often reveal the organism, and some observers maintain that it is as reliable for diagnosis as puncture of the spleen. Examination of ordinary blood-films will sometimes reveal the parasite as first pointed out by Christophers (1904), but many films may have to be examined before a single parasite is seen. In some cases, however, they seem to have been easily found in the peripheral blood.

Thus, Donovan (1905, 1909*a*) states that he had found parasites in the finger blood of over 93 per cent. of the cases in Madras, while Patton (1907, 1912*a*), in the same place, had positive results in thirty-eight out of forty-five cases. Nicolle and Comte (1908*a*) in Tunis demonstrated leishmania in the peripheral blood of a case of the Mediterranean disease, and Cannata (1913–1914) in Italy found them in fifteen out of sixteen cases after examination of many films from each, an observation which was confirmed by Vaglio (1914), who was successful in eleven cases. Knowles (1920) has examined cases from this point of view at Shillong in Assam, with the following results: Seventy-three cases were examined, and parasites discovered in the blood of thirty-three. From the seventy-three cases, 682 films were scrutinized, and sixty-seven of these were positive, revealing 2,839 parasites. It will thus be seen that diagnosis by direct examination of the peripheral blood is not always a simple matter, but usually necessitates the careful and prolonged study of many films. Knowles and Das Gupta (1924*a*) have demonstrated parasites in 67 per cent. of seventy cases by the use of thick films of the peripheral blood.

Mayer and Werner (1914) and the writer (1914) demonstrated the possibility of diagnosis by culture of the peripheral blood. Blood taken from the finger with due care to avoid bacterial contamination is inoculated to a series of tubes of N.N.N. medium, a few drops being added to each tube. Flagellates develop in the tubes after a variable period of two or three weeks. This observation has been confirmed by Row (1914), Giugni (1914, 1914*a*), Cannata and Caronia (1914), Cornwall and La Frenais (1916), and Knowles (1920). Though it is a method of diagnosis worthy of trial, a negative result cannot be held to exclude an infection. Culture of material from spleen or liver puncture should be carried out at the time of film-making, for, when the infection is a slight one, the parasites may be missed in the smears, though sufficiently numerous to develop in the culture.

DIAGNOSIS BY SEROLOGICAL TESTS.—Attempts to obtain a method of diagnosis based on the principle of complement fixation has yielded only discordant results.

Napier (1921, 1922) noted that if a drop of commercial formalin be added to 1 c.c. of the serum of a case of kala azar, the serum solidifies in a few minutes, and very quickly becomes opaque, like the coagulated white of an egg. This reaction, which is called the *formol gel test* or *aldehyde reaction*, is fairly constant in kala azar. It occurs only partially in tuberculosis, leprosy, and heavy malarial infections, and disappears progressively during the course of treatment of cases of kala azar by means of tartar emetic.

Bramachari (1920) described as the *globulin precipitation test* a reaction

which occurs with the serum of kala azar cases. If one part of serum is mixed with two parts of distilled water, an opacity is produced. If the water is poured on the surface of the serum, a ring effect is obtained. The test has been elaborated into a quantitive one by Bramachari and Sen (1923).

Wagener (1923) has shown that the injection of alkaline extracts of *Leishmania* from cultures into the skin of rabbits previously rendered sensitive by injections of cultural forms of *Leishmania* produces a local reaction in the form of an erythematous papule, which reaches its height in forty-eight hours, and persists from three to five days. The antigen can be prepared from both *L. tropica* and *L. donovani*, as it is not specific for either parasite. If these results are confirmed, the reaction may be of use for diagnostic purposes. The serological observations made by Noguchi (1924) have been referred to above (p. 399).

MORPHOLOGY.—The parasite *Leishmania donovani*, which is morphologically indistinguishable from *L. tropica*, is a small organism usually circular or oval in outline (Plate IV., 7-10, p. 406). It consists of a mass of cytoplasm covered by a definite membrane. The cytoplasm contains two very characteristic structures, the recognition of which is essential to the identification of the organism. One is the nucleus, and the other the kinetoplast. The former is a more or less spherical body, with a diameter about one-third to a half of the shortest diameter of the organism. It usually lies against the membrane, and is somewhat flattened on this side. The flattening may be so marked that its form is reduced to that of a hemisphere, or even of a thin disc, so that in optical section it is seen as a semicircle or merely a narrow structure lying along one side of the parasite. The extreme flattening of the nucleus often appears to be intensified by the presence of one or more vacuoles in the cytoplasm, which may be so large as to reduce the parasite to the condition of a thin-walled sac. The second structure of importance is the kinetoplast, which is usually seen as a rod lying with its long axis directed towards the nucleus. In preparations it may appear as a small spherical body, but in most cases this is due to its long axis being perpendicular to the slide. In ordinary dried films stained by the Romanowsky method, the nucleus appears as a mass of bright red granules, while the kinetoplast, which is a more solid compact body, takes a deep reddish-purple tint. In deeply stained parasites a red line, first described by Christophers (1904), can be traced from the blepharoplast, which lies near the centre of the kinetoplast, to the surface of the parasite. This is the axoneme, which gives rise to the flagellum of the leptomonas forms which develop in cultures (Plate IV., 6, p. 406). The size of the parasite varies considerably. When spherical, it measures from 1 to 3 microns in diameter. More usually it is ovoid, with the long

PLATE IV.

***Leishmania tropica* AND *L. donovani* FROM CASES OF ORIENTAL SORE AND KALA-AZAR: DRIED FILMS STAINED WITH ROMANOWSKY STAINS. (× 2,000).**

1. Portion of a field in a smear from an oriental sore, showing *L. tropica* scattered as a result of rupture of an endothelial cell.
2. Detached portion of cytoplasm of endothelial cell showing *L. tropica*. The outlines of the parasites are not visible. Such bodies have been interpreted as schizonts.
3. Red cell with superimposed *L. tropica*.
4. Detached portion of cytoplasm of endothelial cell with *L. tropica*.
5. Large endothelial cell packed with *L. tropica*.
6. Three parasites (*L. tropica*) showing axonemes.
7. Portion of a spleen smear showing *L. donovani*.
8. Detached portion of cytoplasm of endothelial cell in peripheral blood-film of kala-azar case showing *L. donovani*.
9. Large endothelial cell in peripheral blood-film of kala-azar case with a single parasite (*L. donovani*) in the cytoplasm.
10. Group of nine parasites (*L. donovani*) in smear from cervical lymphatic gland of kala-azar case.

(ORIGINAL.)

PLATE IV.

diameter from 2 to 5 microns and the shorter 1·5 to 2·5 microns. In these forms one end is often more rounded than the other. Occasionally, more elongated forms somewhat resembling a cigar or torpedo in shape occur, and in these the kinetoplast may be so closely applied to the nucleus as to escape recognition (Fig. 192). In the smear of a child's spleen made by the writer in Malta, the majority of the parasites were of this type. Occasionally, larger parasites occur, especially in inoculated animals, where they may attain a diameter of 8 or 9 microns. In a spleen smear from a typical case of kala azar there occur numbers of large cells, the macrophages, some of which are packed with parasites. Many of these cells will have broken down in preparation of the smear, and the liberated parasites will be scattered amongst the débris. Detached portions of the cytoplasm of these cells containing groups of leishmania have been called "gangues" by French writers (Plate IV., 2, 4, 8, p. 406). In less heavily infected cases a careful examination of the films will have to be made, as

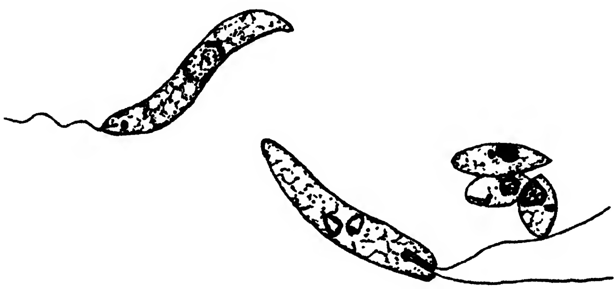


FIG. 189.—FLAGELLATE FORMS OF *Leishmania donovani* IN THE TISSUES OF AN EXPERIMENTALLY INFECTED DOG (× *ca.* 2,000). (AFTER WENYON, 1915.)

the parasites may be present in very small numbers. A group of two or three parasites, or even single ones, will be found in the cytoplasm of a small percentage of the cells. In such cases, careful attention must be paid to the morphology, and no structure should be called a leishmania unless the sharp outline, the deeply staining rod-like kinetoplast and the more palely staining and larger nucleus have been clearly seen. The crucial test in any doubtful case is the development of the flagellate leptomonas form in culture. In every film, in addition to the parasites which show the typical structure, there occur abnormal or degenerate types, about the nature of which it is often impossible to form an opinion.

As seen in dried smears stained by the usual Romanowsky methods, the nucleus appears as an aggregation of red-staining granules. This is an artificial picture, for in films which have been fixed, without drying, in a suitable fixative, and stained by the iron-hæmatoxylin method, the nucleus is seen to have a membrane enclosing a clear space, at the centre

of which is a spherical karyosome (Fig. 192, 7–8). The kinetoplast is a compound body consisting of a rod-shaped parabasal and a blepharoplast from which the axoneme arises. In dried films the axoneme is often seen as a red line after deep staining with Romanowsky stains.

There is no evidence that the leishmania exist in any other than the typical form in the infected host, with the single exception recorded by the writer (1915*a*) of the occurrence of leptomonas forms, such as appear in cultures, in the spleen of a dog infected with leishmania from a case of Indian kala azar (Fig. 189). In this animal the leishmania were of a particularly large size and varied shape.

Maitra (1924), in India, found in a peripheral blood-film, made from a case which was clinically one of kala azar, flagellates which appeared to be of the leptomonas type. Subsequent examinations of the blood did not reveal any flagellates, so that it is not improbable that the film had been contaminated. The writer knows of an instance in which similar flagellates were deposited on a blood-film by a fly. There is nothing in Maitra's account to suggest that such a contamination took place, but, from information the writer has received, such a fallacy was not excluded, for the flagellates were only found in one of several films made at the same time.

MULTIPLICATION.—The only method by which *Leishmania donovani* multiplies is by binary fission. Dividing forms with two nuclei and two kinetoplasts, and these structures actually in process of division, can easily be found in stained films. The minute details of the division process can only be followed in properly fixed films. In dried films stained by Romanowsky stains, the red mass representing the nucleus elongates, becomes dumb-bell-shaped, and then divides into two parts. The kinetoplast divides by elongation and division of the blepharoplast, followed by a similar process in the parabasal. After division of the blepharoplast, a new axoneme is formed from that daughter blepharoplast, which is not attached to the old axoneme. In dividing leishmania, it is sometimes possible to distinguish two parallel axonemes arising from an incompletely divided kinetoplast.

Multiple segmentation has been described by several observers (Mackie, 1914, Yakimoff, 1915*a*). The evidence rests on the appearance in films of cytoplasmic bodies within which are arranged a varying number of nuclei and kinetoplasts, without any outlines to indicate separate organisms. In the writer's experience, these bodies probably represent detached portions of cytoplasm of the large cells containing leishmania, of which the outlines are not clearly visible, either as a result of imperfect staining or degenerative changes undergone by the parasites (Plate IV., 2, p. 406). Similar appearances are often seen when the large cells are still

intact, and where the cytoplasm is dotted over with pairs of nuclei and kinetoplasts, just as they are in the supposed multiple segmentation forms. Still more doubtful are the forms which Archibald (1913, 1914), Smallman (1913), and Statham and Butler (1913) have described. These are more or less spherical portions of cytoplasm containing granules, which have no such definite arrangement as the nuclei and kinetoplasts in the bodies just discussed. They bear some faint resemblance to schizogony stages of malarial parasites or other organisms as seen in dried smears. Here, again, the origin of these structures is in the cytoplasm of large cells with granular cytoplasm, portions of which have been broken off. They are merely fragmentation bodies, and have no relation to the leishmania.

CULTURE.—The greatest interest attaches to the culture of leishmania. Rogers (1904) demonstrated that flagellates of the leptomonas

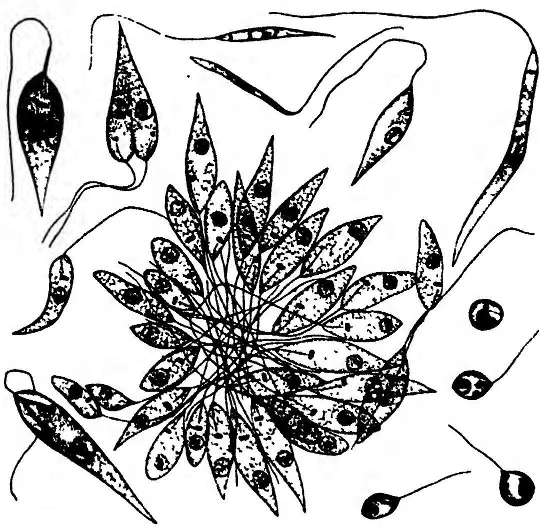


FIG. 190.—CULTURE FORMS OF *Leishmania donovani* FIXED WITH SCHAUDINN'S FLUID AND STAINED WITH IRON HÆMATOXYLIN (× 2,000). (ORIGINAL.)

type appeared in citrate solution, to which material from spleen puncture of cases of kala azar had been added, an observation which proved conclusively the flagellate nature of the puzzling Leishman-Donovan body (Fig. 190). Though flagellates developed and multiplied in the medium employed at the ordinary laboratory temperature, this was not always the case, and subculture was not satisfactorily obtained. Rogers obtained better results with citrated human blood acidified with citric acid, but it was Nicolle (1908*c*) who demonstrated the possibility of culture at a temperature of 22° C. in the water of condensation in tubes of Novy and

McNeal's rabbit blood-agar, and in a simplified medium now known as the N.N.N. (Novy, McNeal, Nicolle) medium. Furthermore, in this medium subculture was readily obtained, so that the flagellate could be maintained as easily as any bacterial organism. Since that time the culture method has been universally adopted, and is a recognized aid to diagnosis. A strain of *L. donovani* isolated in 1910 was reported by Nicolle (1925) to be still growing in N.N.N. medium. During these years it has been subcultured 395 times.

The change undergone by the leishmania when introduced into the medium can be studied in heavy infections by examination of the inoculated material at frequent intervals. According to Leishman and Statham (1905), who studied the development, the first change is an increase in size of the leishmania, a growth of the nucleus, and an increased vacuolization of the cytoplasm. In many cases the leishmania become pyriform in shape. After forty-eight hours, growth of the flagellum commences. It is a rapid process, and takes place from an eosin staining vacuole or body which lies in front of the kinetoplast. This body moves to that end of the organism which will be its anterior end in subsequent development. Its contents are extruded as a series of fine filaments, which unite and form the rudiments of the flagellum. As this process was studied only in dried films, it is probable that the appearances are artefacts due to a rupture of the vacuole. It is far more probable—and this is supported by what is known of the formation of flagella in other organisms—that the axoneme, which is sometimes visible in the leishmania, continues its growth, and extends through the surface of the parasite to form the flagellum. Active flagellate forms may be seen in cultures at any time between forty-eight and seventy-two hours after the medium has been inoculated. Very soon many of the organisms become still more elongated till the body measures from 10 to 20 microns in length, while the flagellum is often longer than the body. The fully-formed flagellate is flattened like a blade of grass. Sometimes one edge of the organism is convex and the other slightly concave, giving it the shape of a curved sword-blade. In a culture of four or five days' growth various types of flagellates are present, including round forms 4 to 5 microns in diameter with long flagella, broad pyriform individuals with rounded anterior and tapering posterior ends, and measuring about 10 microns in length and 4 to 5 microns in breadth, and the longer sickle-shaped forms already mentioned (Fig. 190). The various types are connected by intermediate forms. Reproduction by longitudinal division takes place rapidly till the culture at the end of a week to ten days may be swarming with flagellates. At division the blepharoplast divides, and a new axoneme is formed by outgrowth from the blepharoplast. The parabasal then becomes constricted

and divides, and at about the same time nuclear division commences. In properly fixed material the nucleus is seen to elongate, while the spherical karyosome at its centre elongates also, and is finally divided into two parts, after which the entire nucleus becomes constricted at its equator, and finally two result. Schulz (1924) maintains that the nucleus divides by mitosis. Meanwhile, the new axoneme has continued its growth, and a new flagellum is formed which gradually increases in length. Though multiplication of the flagellum by longitudinal division has been described, it is extremely doubtful if such a process ever occurs. After the new flagellum has developed, splitting of the body, whether in the rounded or elongated form, commences between the two flagella, and extends in a posterior direction till two flagellates result. A characteristic feature of the cultures is that the flagellates tend to remain clustered in groups, with their flagella directed towards one another, so that rosettes or spheres of organisms are formed with the flagella entangled at the centre. As the cultures become old, elongate forms become less numerous, and many rounded, non-flagellate bodies appear which resemble in many respects the original leishmania. Many of these are, undoubtedly, degenerate or dead forms, but the fact that subculture can often be obtained from cultures of this type proves that some of them, at any rate, are living. Cultures can be obtained from the spleen, as first shown by Rogers (1904), or any other organ in which the parasites occur. They were cultivated from the blood by Mayer and Werner (1914), and by the writer (1914), while Shortt (1923*c*), and Shortt, Swaminath, and Sen (1923), have succeeded in growing them from the centrifuged deposit from the urine of three cases of kala azar.

Noguchi has found that *L. donovani* in culture can be differentiated from other species of *Leishmania* by serological tests (p. 399).

NATURAL INFECTIONS OF ANIMALS.—The only animals which have been found naturally infected with *L. donovani* are dogs and cats, and the latter only on one occasion, when Sergent Ed. and Et., Lombard, and Quilichini (1912) published an account of a case of kala azar on a farm near Algiers. The infected child was associated with a dog and a kitten about four months old, both of which were infected.

The dog has been frequently found infected, especially in the Mediterranean region, and often in association with infected human beings. This has given rise to the view of the canine origin of kala azar.

The natural disease in dogs may run an acute or chronic course, and the symptoms, as in man, are loss of weight, fever, anæmia, enlargement of the liver and spleen. The dogs appear in bad condition, and are mangy and often die of intercurrent infections. Recovery takes place more frequently than in human beings.

The first observation of canine kala azar was made by Nicolle and Comte (1908) in Tunis, an endemic centre of the human disease. A large number of examinations were subsequently made in Tunis and other parts of Lybia by Nicolle, the Yakimoffs (1911*c*), Gray (1913), and others, with the result that the ordinary street dogs were found infected to the extent of about 1·6 per cent. Examining a series of dogs which were evidently in bad condition, Nicolle (1914) found a percentage of 5·5 infected. Similar observations were made by Sergent Ed. and Et. (1910), Senevet (1912), and Lemaire, Sergent, and Lheritier (1913) in Algiers, where the human disease also exists. The canine disease has also been seen in parts of Africa where the disease in man is rare or unknown. Thus it has been found in Morocco by Delanoë and Denis (1916), and at Dakar (Senegal) by Lafont and Heckenroth (1915). In the Sudan, Bousfield (1911) found bodies somewhat resembling leishmania in a dog, but Archibald (1914) examined many dogs in the endemic area without encountering a single case of the canine disease. In endemic centres in Europe the disease in dogs has been frequently encountered. In Malta, Critien (1910 and 1911) found three out of thirty dogs infected, and the writer (1914*a*) six out of forty-six. Alvarez and Pereira da Silva (1910, 1911) examined 300 dogs in Lisbon and found eight infected, and in a later series four out of 109. Martinez (1915) discovered the first canine case in Spain, while Pittaluga (1914) observed three infected dogs in Tortosa and Beninar. In Italy and Sicily, where infantile kala azar exists, the canine disease has also been found. At Bordonaro in Sicily, for instance, Basile (1910) claims to have found infection in as many as twenty-seven out of thirty-three dogs examined. In Palermo itself Jemma (1910 and 1912) found no case amongst 227 dogs examined, but in the environs of the town discovered two infected animals, one of which was in close association with a human case. In the same town Caronia and di Giorgio (1914) examined with negative results 1,005 dogs, while in Catania Pulvirenti (1911) saw three infections in a series of 275 dogs. These places in Italy and Sicily are endemic centres of the disease, but canine kala azar has also been found in Rome, which is not an endemic centre, though a single case in a child has been recorded here. The human disease has, however, been recorded from Nice by Labbé, Targhetta, and Ameuille (1918). In Greece, Cardamatis (1912) found eighty-one dogs infected amongst 589 examined in Athens, and Lignos (1913), in the Isle of Hydra, found a percentage of infections of 16·66 from May to October, while later (1916) another series examined during the winter (October to April) gave a percentage of 8·77. In the Trans-Caspian region Dschunkowsky and Luhs (1909*b*) observed cases of canine kala azar, while in Turkestan Kohl-Yakimoff, Yakimoff and Schokhor (1913), Yakimoff and Schokhor (1914), and Yakimoff (1915*a*) found dogs to be infected in a percentage varying from 25 to 35, according to the season. Adelheim (1924) has reported kala azar in a child and a dog in Riga. Both contracted the disease in Tashkent, where the family had been living. Césari (1925) reports the canine disease at Grasse in the South of France.

In India the results have been very different. Donovan (1909*b*), working in Madras, examined 1,150 dogs, 256 of which came from the kala azar quarter of the city, without finding a single infection. Donovan (1913) and Patton (1913) recorded the same result after a further examination of 2,000 dogs, also in Madras. Mackie (1914) failed to find an infection amongst ninety-three dogs examined in the villages of Nowgong (Assam), where the human disease is endemic. On the other hand, Castellani (1912) claims to have observed the disease in several dogs in Colombo, which is not an endemic centre of kala azar in man. Such an anomalous statement can hardly be accepted till confirmatory evidence is forthcoming. Mr. Burgess, of the Bacteriological Institute of Colombo, at the writer's request, kindly made spleen smears from 250 dogs in Colombo. In none of these was the writer able to find leishmania.

RELATION OF HUMAN TO CANINE KALA AZAR.—The important question arises as to whether the naturally occurring disease of dogs is due to *Leishmania donovani* or to some other species. The frequent association of the disease in dogs with human cases in the Mediterranean area, and the morphological identity of the parasites, are facts which make it impossible to regard the organism from dogs as other than *L. donovani.* Furthermore, the disease produced in dogs by inoculation with the parasite from human sources is identical with the natural canine disease, while the organism from the canine disease is inoculable to animals, with results similar to those which result from inoculation of the human virus.

The apparent absence of the canine disease in endemic areas in India has been urged as evidence that the Indian disease is distinct from the Mediterranean. The Indian disease is, however, inoculable to dogs, so that the freedom of the Indian dog from infection probably depends on some factor not at present understood. In the present state of knowledge, and lack of absolute proof of the method of transmission of the disease, it is better to consider all the various systemic diseases in man and dogs as due to one parasite, *L. donovani.* It is hardly necessary to again remark that morphologically (in smears and cultures) the parasites from the various sources are identical.

Though it is admitted that the human and canine diseases are caused by the same organism, this does not mean that the dog is to be regarded as a reservoir of the virus. Some have maintained that in Italy the disease necessarily passes from dog to man, but so many cases occur which cannot be associated with any infected dog that it would appear that the infection of the animal is as much an accident as the infection of the human being. Areas occur in which, apparently, only dogs have the disease, while in others only human cases are known. It is claimed, however, by Basile (1916) that in Bordonaro in Sicily, where a high percentage of naturally infected dogs occurred, the extermination of these has led to an almost complete disappearance of the human disease.

SUSCEPTIBILITY OF ANIMALS.—Nicolle (1908*a*, 1909*a*), and Nicolle, Comte, and Manceaux (1908), were the first to show that *L. donovani* of Mediterranean origin was inoculable to dogs and monkeys. The failure to produce infection in animals by observers in India was advanced as a proof of the existence of two species of leishmania in kala azar. It is now known that the Indian virus, if injected in sufficiently large doses, will give rise to infections as often as the Mediterranean virus. Infection is produced most readily by intraperitoneal inoculation of large doses of the material obtained by crushing an infected spleen, liver, or bone marrow in normal saline solution. In larger animals, inoculation can be made intrahepatically or intravenously. Subcutaneous inoculation does not

produce infection so readily. Animals may be infected by injection of large doses of the cultural forms, but infection is less likely to take place than after a dose of the virus from the organs of man or another animal. Organisms which have been maintained by subculture for long periods are less liable to infect than those more recently isolated.

The infected animals often recover, and, as has been clearly demonstrated by Laveran, on the passage of the virus from animal to animal it loses its virulence to such an extent that finally infection does not occur. This is equally true of the mouse, dog, and monkey—the animals which have been used to the largest extent—though from the recent observations of Young, Smyly, and Brown (1924) in North China, the hamster appears to be more susceptible. The majority of animals, if young when inoculated, continue to increase in weight in spite of their infection, though subject to minor disturbances of health such as slight attacks of fever. In some cases the infection is more acute, and after a rapid loss of weight death occurs.

The infection produced in experimental animals is of slow development, and cannot be compared with that resulting from the inoculation of pathogenic trypanosomes. The lack of a suitable experimental animal has been a great handicap to investigation work.

In some cases, by the inoculation of the skin, observers have been able to produce with *L. donovani* local cutaneous lesions resembling oriental sore.

Shortt (1923*b*) inoculated a number of caterpillars and other invertebrates with cultures of *L. donovani*. In the case of one caterpillar, active single and dividing flagellates were found in the body cavity fluid a week later.

The Mediterranean virus has been successfully inoculated into dogs by several observers since Nicolle's first success in 1908. Jemma, di Cristina, and Cannata (1910) were successful in Italy, and Novy (1908) in America with a culture which had been sent to him. Nicolle and Blaizot (1912) proved that the jackal was also susceptible. Yakimoff (1915*a*), working in Turkestan, succeeded in infecting dogs and mice with the local virus. The most extensive series of experiments with dogs has been made by Laveran (1917), who employed a virus obtained in Tunis. Of thirty dogs inoculated with material from the organs of infected animals, twenty-six became infected. In five dogs which died of the disease, the average duration was 257 days. Two dogs killed on the 454th and 456th days were still found infected, though the condition of the organs showed them to be on the road to recovery. Nicolle and Laveran have both noted keratitis in infected dogs. The Indian virus was first inoculated to dogs by Donovan (1913). At about the same time Patton (1913) was also successful with the dog and jackal. The writer (1914*a*, 1915*a*) in London infected a dog from a case of kala azar from India. Subsequently the virus was passed through four successive dogs, when the inoculations were discontinued. Mackie (1915*b*) also succeeded in infecting dogs with the Indian virus. Laveran (1913, 1917), commencing with a culture of *L. donovani* obtained from Row in India,

inoculated twelve dogs either intravenously or intrahepatically. Five of these became infected. Two others were infected by inoculation with both cultures and spleen material from a heavily infected monkey. The course of the disease resembled that produced by the Mediterranean virus. In two cases keratitis was observed.

Nicolle (1909) succeeded in infecting monkeys (*Macacus sinicus* and *M. cynomolgus*) with the Mediterranean virus in Tunis, while Laveran (1917), with the same virus, inoculated fourteen monkeys (*M. sinicus*, *M. cynomolgus*, and *M. rhesus*), of which two acquired a fatal infection, seven only a slight one, while five did not become infected. Marshall (1911), working in the Sudan, succeeded with five monkeys (*Cercopithicus sabæus*) out of seven inoculated with the Sudan virus. Archibald (1914) also infected a monkey of the same species. Monkeys infected may die in a couple of months, or the disease in them may run a chronic course terminating in recovery. The infection shows the same irregularities as in the dog. With the Indian virus, Row (1912) produced a general infection in *M. sinicus* and *M. cynomolgus*. Of especial interest are the results obtained by this observer after local inoculation of the skin. A *M. sinicus* was inoculated with material from the spleen of a case of kala azar by scarification of the skin, and another by subcutaneous injection of a culture. In both cases local nodules appeared at the sites of inoculation several months later. Leishmania were present in these nodules, one of which was excised and used for further inoculations. With the material thus obtained another monkey was inoculated in the skin, with the production of a local infection, while two mice and a monkey injected intraperitoneally acquired a general infection. Experiments of a similar kind were carried out by Korke (1914). He noted, however, that subcutaneous inoculation sometimes gave rise to a local skin lesion, and at others to a generalized infection. In some cases where a local lesion was produced, a generalized infection occurred at the same time. Tyzzer and Walker (1919) produced a purely local lesion by inoculating a monkey cutaneously with cultures of the Mediterranean virus. In this case, there was an incubation period of four months. Laveran (1913, 1917), working with the Indian virus, infected monkeys in Paris, and found the course of the infection similar to that produced by the Mediterranean virus. Shortt (1923*b*) inoculated thirteen monkeys with virus from man or other monkeys, and obtained infection in ten. Laveran and Pettit (1909*a*) were the first to produce an infection in mice with the Mediterranean virus. Successful results were also obtained by Yakimoff and Kohl-Yakimoff (1912) and Rutelli (1914) in Italy. Continuing his experiments, Laveran (1920) noted that the virus could be handed on from mouse to mouse, but that the depreciation in virulence was very marked. He gives the following results of the passage of a strain of *L. donovani* of canine origin through mice:

	Number of Mice Infected.	*Not Infected.*	*Recovered.*
1st inoculation	17	1	7
2nd ,, ...	13	2	8
3rd ,, ...	16	3	8
4th ,, ...	19	0	15
5th ,, ...	14	1	9
6th ,, ...	6	0	5

It thus appears that with successive passages through mice, though infection usually takes place, the percentage of natural recoveries increases. The infected mice became anæmic, showed marked enlargement of the spleen, and degeneration of the testicle. Mice examined over a year after inoculation were in some cases still infected, while in others, though no parasites could be found, the characteristic lesions were still present.

With the Indian virus, Row (1912, 1913) was successful in inoculating mice. The virus employed was obtained either from a local cutaneous nodule in a monkey, the spleen of infected monkeys, or cultures of the organism. Mackie (1914, 1915*b*) infected mice from material from human cases, and Laveran (1917) was also successful. Shortt (1923*b*) produced a heavy infection in a mouse with the Indian virus. Adelheim (1924) produced heavy infections in mice with a virus he obtained from a dog which had been brought to Riga from Tashkent. Subcutaneous injection produced, not only a generalized infection, but also a local sore in which the parasites tended to persist longer than in the internal organs.

Rats have also been infected with the Mediterranean virus by Laveran (1912*c*) and Yakimoff and Kohl-Yakimoff (1912*a*). Patton (1912*a*) infected a rat with the Indian virus, while Cornwall and La Frenais (1916) infected one by injection of cultures and another by feeding it on bread soaked in culture. The organisms were only demonstrated in the infected animals by the culture method. The writer (1915*c*) was also successful in infecting white rats directly from a human case.

Guinea-pigs were first shown to be susceptible to the Mediterranean virus by Laveran and Pettit (1909*b*). Franchini (1911) claimed to have produced a general infection in a young guinea-pig by injection of cultures. With the Indian virus guinea-pigs have not yet been infected. The only general infection in a rabbit was recorded by Mantovani (1912). Volpino (1911) infected the cornea of a rabbit by scarification with material from the spleen of an infected dog. About three months after the cornea showed a lesion which resembled those produced by the virus of syphilis. Numerous leishmania were present in the lesion. Rabbits do not appear to have been infected with the Indian virus. Rabbits and guinea-pigs are evidently difficult to infect, as many failures have been recorded. Cats also have never been infected, though on one occasion a naturally infected kitten was discovered in Algiers.

With the Sudan virus Archibald (1914) infected the jerboa and the gerbil, while with the Indian virus Mackie (1914) infected the flying fox (*Pteropus edwardsi*).

In this connection it is of interest to note that Archibald (1914), working with the Sudan virus, was successful in infecting two monkeys by feeding them with crushed infected spleen of man or experimental monkey. He failed to infect a young dog by this method.

As regards infections in animals, it is rarely that leishmania can be found in the peripheral blood. Liver puncture can generally be carried out, but parasites are not numerous in this organ. Spleen puncture is difficult to perform, though on one occasion the writer diagnosed a case of infection in a dog by this method in Malta. Bone marrow can be obtained by trephining a rib or one of the long bones of the leg under an anæsthetic. In dogs, at any rate, this method gives the most reliable information. Cultures from the blood have also been obtained.

Dogs and monkeys which have recovered from infections have been shown by Nicolle (1910) and Nicolle and Comte (1910) to be immune to further inoculations. Laveran (1914*a*) notes that a monkey which had recovered from infection with the Mediterranean virus was immune to inoculation with the Indian one.

From the foregoing summary it will appear that many successful inoculations of animals have been effected. The infections, however, cannot be compared with those produced by pathogenic trypanosomes, for they are nearly always of slow development, and the number of organisms found is generally small. The want of an easily inoculable and susceptible host for *L. donovani* has been a great obstacle to the carrying out of experimental work on the method of transmission of kala azar. Recently, however, Smyly and Young (1924), and Young, Smyly, and Brown (1924), have shown that in North China the hamster (*Cricetulus griseus*) is more susceptible than other laboratory animals. Successive passages of a virus were

effected, and as it showed no signs of becoming attenuated, it is possible that this animal may prove useful for experimental purposes, and lead to important results on the ætiology and transmission of kala azar. Meleney (1925) has shown that the infection progresses steadily in its intensity till, at the end of fifteen months, the tissues of the spleen, liver, bone marrow, lymphatic glands, and intestinal mucosa have been largely replaced by macrophages packed with parasites. The parasites are found also in other organs, including the meninges, where the macrophages occur. They were also demonstrated in the glandular cells of the liver.

Franchini (1922*m*) claims to have produced infection of the plant *Euphorbia ipecacuanha* by inoculating it with cultures of *L. donovani*.

TRANSMISSION.—Since Rogers's demonstration of the development of flagellates of the leptomonas type from leishmania, and the recognition of the close resemblance of these to natural insect flagellates, it has been generally assumed that *Leishmania donovani* has an invertebrate host. Though many attempts have been made to discover such a host and the method of transmission of kala azar, the problem still remains unsolved. Many different invertebrates, chiefly bugs and fleas, have been considered, and some observers claim to have effected transmission of infection by the agency of fleas. As leishmania are present in the peripheral blood of cases of kala azar, they are readily ingested by blood-sucking insects, while the flagellate forms which appear in cultures undoubtedly represent an insect developmental phase, as they do in cultures of trypanosomes, the invertebrate hosts of which are known in many cases. It was suggested by the writer (1914*a*) that oriental sore and kala azar may be caused by insect flagellates which only accidentally infect man. Normally, the flagellates would pass from insect to insect, as do all naturally occurring insect flagellates. Occasionally, they would infect human beings, and give rise to the diseases mentioned. According to this view, the virus could be maintained indefinitely in the insects, which would be infected from one another, though an insect would be capable of infecting itself by sucking the blood of an infected human being.

As first demonstrated by Patton (1912*a*), it is well known that *L. donovani* will develop into the leptomonas form in the stomach of the bed bug. The flagellates can be recovered from the intestine of the bug by the culture method as long as six weeks after parasites were first ingested. Mice can be infected with the forms in the intestine of the bug nine days after the feed on kala azar cases. An enormous amount of time and energy has been spent in investigating the claims of the bed bug, but no actual proof that it is the transmitter of kala azar has been obtained. Recently, Knowles, Napier, and Smith (1924), Christophers, Shortt and Barraud (1925, 1925*a*), and Shortt, Barraud and Craighead (1926) have found that female *Phlebotomus argentipes* acquire a heavy leptomonas infection of the intestine and pharynx after feeding on kala azar cases.

The flagellates may even extend into the buccal cavity. This fact, combined with Sinton's observation that the distribution of the disease in India corresponds with that of *P. argentipes*, leaves only a definite transmission experiment to prove that kala azar is conveyed by the bite of the sand fly. The claim that the flea is the transmitter of kala azar in the Mediterranean area has not been substantiated. If it be assumed that an insect vector exists, then there are two possibilities as to the mode of infection. The organism may either be injected by the insect by way of the proboscis (inoculative), or it may be voided in the fæces of the insect in some form, and thus infect the wound or be ingested (contaminative).

It has been suggested that *L. donovani* may escape in the fæces of patients. Manson and Low (1904) demonstrated its presence in the ulcers of the intestine, while Perry (1922) has seen the villi heavily infected with parasites. Mackie (1914*c*) saw bodies resembling leishmania in mucus from the intestine, but Knowles (1920) examined mucus very carefully, and though he saw bodies more closely resembling leishmania than those noted by Mackie, he pronounced no opinion as to their nature. It seems probable that these bodies are yeasts, which frequently show a striking resemblance to leishmania in stained films. Shortt (1923) has cultivated leishmania from the urine of kala azar cases, so that the possibility of spread of infection by water has to be considered, but experience has shown that the parasites quickly degenerate in water. The nature of the parasite is not in favour of such a method of transmission, though Adelheim (1924) has noted that a healthy mouse kept in a jar for five months with an infected mouse contracted the disease. As the infected mice commonly had ulcers in the intestine in which parasites could be demonstrated, it was thought that oral contamination was responsible for this contact infection.

The common association of ankylostomiasis with kala azar has suggested the possibility of the ankylostomes being a source of infection. Knowles (1920) investigated the worms taken from kala azar cases, and even the eggs and embryos hatching from them, without finding anything to support this view.

The following experimental work with insects has been carried out with a view to the discovery of a transmitting host:

Bugs.—The bed bug (*Cimex rotundatus*) was suspected by Rogers as a possible carrier of kala azar on account of its frequent presence in houses where cases of the disease occurred. Patton (1912*a*), working in Madras, also favoured this view, and conducted a series of experiments by which he claimed to have proved the correctness of the theory. By feeding bugs on cases of kala azar, in which the leishmania were numerous in the peripheral blood, and dissecting them at varying intervals, he found that the leishmania had developed into flagellates of the leptomonas form as they do in cultures, and that some multiplication had taken place.

In bugs dissected eight to ten days after feeding, the flagellate forms had given rise to rounded bodies again. The various stages were compared with the similar natural flagellates of insects, and were described as pre-flagellates, flagellates, and post-flagellates. It was found, however, that the flagellates did not persist in the bug, and, furthermore, it was noted that a second feed of blood often caused the organism to disappear more quickly. The writer (1912*c* and 1915*b*) criticized the conclusions drawn from these experiments, and pointed out that the development which took place in the bug was probably due to the large quantity of blood in the stomach, and that it represented merely a temporary culture as occurred in the test-tube. This view received support from the fact that a development of *Trypanosoma lewisi* would take place in the bug, which cannot be considered to be a host for this flagellate. Patton was never able to demonstrate actual transmission by bed bugs. Mackie (1914 and 1915) published an account of further investigations with the bed bug. He dissected over 1,500 bugs from kala azar areas without finding a single one infected. Two monkeys were inoculated with 209 and 606 crushed bugs without becoming infected. Young bugs born in the laboratory to the number of 131 were fed on kala azar cases. In only two dissected twenty four hours after feeding were leishmania seen. On another occasion, 191 young bugs gave a negative result. Cornwall and La Frenais (1916) succeeded in causing bugs to ingest cultural forms of *L. donovani*. The bugs were then fed on rabbits. It was found that in some cases the flagellates multiplied and persisted up to twenty-nine days. Attempts were made to infect citrated rabbit's blood by causing these bugs to bite through skin. The blood was then distributed in N.N.N. medium. In no case was a culture of flagellates obtained. The fæces of the bugs never contained encysted forms of the flagellate such as are found in the fæces of insects with a natural flagellate infection. Rounded forms and flagellates were, however, seen in the rectum, but these appeared to be in process of degeneration. A peculiar type of organism, called the "thick-tailed form," was seen in the bugs. This consists of a rounded body of the usual leishmania structure measuring 5 to 6 microns in diameter, and provided with a long flagellum which is very much thicker than that of the ordinary flagellated forms. It is thus apparent that the bed-bug hypothesis has not been established, and no proof has yet been given that the development which takes place in the bug is other than a temporary culture of the organism. This is all the more probable from an account of investigations made by Patton, La Frenais, and Rao (1921) in Madras. By feeding bugs on material containing *Leptomonas pulicis*, *Crithidia ctenocephali*, and *Herpetomonas muscarum*, and making cultures in N.N.N. medium from the alimentary tracts of the bugs at varying intervals, it was shown that these flagellates persisted for twenty-four, eight, and forty-five days respectively. By similar experiments made by feeding bugs with cultures of *Leishmania tropica* and *L. donovani*, these authors (1921) obtained cultures from bugs after forty-four and forty-one days respectively. Shortt (1923) has also obtained active multiplication of *Leptomonas ctenocephali* in the intestine of bugs fed on cultures.

A series of experiments with bugs was conducted by Adie (1921) in India. Many attempts were made to obtain a satisfactory development of *L. donovani* in the bed bug, but without result. Finally, some bugs which had died after being fed on spleen puncture material from a case of kala azar were placed in saline solution in the incubator at 27° C. These were examined about thirty-six hours later, and in one there were found numerous developmental forms of leishmania. These not only occurred in the lumen of the gut, but also in the intestinal cells in clusters, which are compared with the intracellular stages of development of *T. lewisi* in the flea. Apparently, similar stages were not found in the numerous live bugs dissected, so that it would seem that here, again, the development was of the cultural type, and

had taken place within the cytoplasm of dead cells, just as it does in a culture medium. The figures purporting to illustrate the development are not convincing, and suggest the possibility of a mixed infection of leishmania and some other parasite, such as a Sporozoon.

Patton (1922) states that by a special technique he has confirmed Adie's observations. He does not describe the technique, but, presumably, it is the culture of leishmania in the bug's intestine after removal from the body. Neither Patton nor Adie was able to obtain the intracellular development in living bugs. Because multiplication takes place in dead or dying cells, it is not legitimate to conclude that it will also occur in living ones.

Cornwall and La Frenais (1922) repeated these experiments with living bugs. They fixed the intestines entire and examined them in serial section, so as to retain the normal relations of the cells. Though developmental forms of leishmania occurred in the lumen of the intestine, sometimes in enormous numbers, there was no indication of any intracellular development.

The bodies which Adie (1922, 1922*a*) saw in the salivary glands of bugs, and which were regarded as leishmania, have proved to be spores of a microsporidian. Shortt and Swaminath (1924) have tested the infectivity of the developmental forms of *L. donovani* in bed bugs which had fed on a case of kala azar with parasites in the peripheral blood. Nine days after feeding on the case the bugs were dissected, and emulsion of their intestines injected intraperitoneally into mice. In the case of one of these animals a culture was obtained from the spleen 123 days after the injection. No parasites could be discovered in smears of the organs. Thus the forms in the bugs on the ninth day were infective to mice, so that, as these intestinal forms are presumably passed in the bug's dejecta, it is possible they might be ingested or contaminate the puncture wound during or after feeding. Nicolle and Anderson (1925) using over 2,000 bugs in Tunis failed to transmit *L. donovani* from dog to dog, while Shortt and Swaminath (1925) were equally unsuccessful in similar experiments with monkeys in India.

Conorhinus rubrofasciatus.—Donovan (1909*a*) suggested this bug as a possible vector of *L. donovani*, but no evidence of the development of the parasite in this bug could be obtained by Patton (1912*a*).

Fleas.—The occurrence of kala azar in dogs naturally turned the attention of investigators to the possibility of fleas acting as transmitters. This view was first expressed by Nicolle (1908*d*), and was investigated by Basile in Italy (1910*a*, 1911, 1911*a*), who published the results of a series of observations by which he claimed to prove that fleas were the true hosts of *L. donovani*. It has already been remarked that at Bordonaro in Sicily, an endemic centre of the human disease, a high percentage of dogs was found to be infected. According to Basile, the only ectoparasites common to dog and man are the fleas *Pulex irritans* and *Ctenocephalus canis*. Attention has been drawn above to the fairly frequent association of infected human beings and dogs in the same house.

Basile's investigations were conducted on two lines—namely, attempts at infection of healthy dogs by fleas and the study of the leishmania in the flea. In the first place (1911), he claimed that fleas which fed on spleen juice of cases of kala azar became infected with cultural forms of leishmania, and that these produced infection when injected into dogs. Three dogs were then said to have acquired the disease by causing them to live with an infected dog in Bordonaro. In another experiment four dogs were infected in Rome by placing on them fleas taken from infected dogs in Bordonaro. Experiments of this kind were repeated, and from what is now known of the difficulties attending the inoculation of animals it is remarkable with what apparent ease positive results were obtained. Sangiorgi (1911) relates that he

received in Turin an infected dog from Tunis. This dog was placed in a kennel with another dog, unfortunately not examined, which was afterwards found infected. The brothers Sergent, Lheritier, and Lemaire (1912) allowed a pup, previously examined and found uninfected, to be bitten eighty-two times by fleas fed one to eight days previously on an infected dog. The pup was later found infected. Care had been taken to keep the pup free from ectoparasites. The experiments of Basile, apart from any doubt one may have on account of his uniformly successful results, may be criticized from the point of view of the difficulty of excluding with any degree of certainty a previous infection in these animals, though the author claims to have done this. During the long incubation period of a leishmaniasis it is almost impossible to exclude other sources of infection. The same remark may apply to the experiment of Sergent and his co-workers. In many cases it requires exhaustive study and examination to detect a small infection in an animal when it has been killed, but the difficulty is increased a hundredfold when the animal is alive, and reliance has to be placed on puncture of the organs. It is almost impossible to perform a spleen puncture on a dog during life, while liver puncture is most unreliable as a means of revealing an infection.

Working in Malta (1914*a*), the writer carried out a careful experiment. Four young dogs were sent from England by sea, and, on arrival in Malta, two were placed in a flea-proof cage and two in an exposed cage near the other. Over 400 fleas were then transferred from an infected dog kept in another part of the town to the flea-proof cage. Within three weeks the dogs appeared evidently ill, and between five and six weeks after exposure to infection they both died within a day of one another. They were very emaciated and anæmic. No trace of leishmania infection could be detected, and the post-mortem appearances were quite unlike those of kala azar in dogs. The organs were very pale, and the spleens reduced in size and almost white. The animals had died of anæmia through abstraction of blood by the fleas, which had multiplied to an enormous extent. The two control dogs remained perfectly healthy.

Basile (1911) first announced that the dog flea fed upon spleen juice containing leishmania became infected with cultural forms of the parasite. It may be remarked that it is extraordinarily difficult to induce fleas to feed on such material. The writer has always failed after many attempts. Basile then claimed to have found flagellate forms of leishmania in the human flea. In criticism of these statements, it was pointed out that fleas were liable to natural leptomonas infections, and Basile then qualified his statements by claiming to be able to distinguish the natural leptomonas of fleas from those forms derived from the leishmania. It was evident he had not excluded the natural infections of the fleas before placing them in contact with spleen juice containing leishmania. For the study of flea infections, Nöller (1912*d*) had introduced a method of controlling fleas by fixing them on fine wire. The writer has employed this method with very good results. Basile stated that he had used this method in one experiment, and that in two days 200 fleas from an infected dog were fixed on wire in this manner. On the third day they were all fed on a newly-born pup, and the fæces they passed while feeding were examined. Three of the fleas were found infected with leishmania. The two more heavily infected fleas were then dissected and the gut contents injected into two mice, one of which was found infected fifty-six days later. The fixing of a flea on wire is a delicate operation which requires much experience, and may take as long as half an hour. The feeding of the flea till it passes fæces on to a cover-glass, which is then made into a film and stained, may occupy ten minutes or as much as an hour. It is inconceivable that Basile tethered 200 fleas and examined them in so short a time. Furthermore, if his object was to discover infected fleas, this could have been more quickly done by simple dissection. The object of Nöller's method is to enable

repeated examination of the fæces of fleas to be made, so that natural intestinal infections can be excluded before employing them for feeding experiments. On account of these and other incomprehensible statements, it seems impossible to accept Basile's claim that he has demonstrated the flea transmission of Mediterranean kala azar. Pereira da Silva (1913, 1915) conducted a very careful series of experiments, employing Nöller's method amongst others, with a view to determining the possibility of *L. donovani* developing in the human and dog flea. He could obtain no evidence whatever of such a development, and came to the conclusion that the flea is not the transmitting agent of kala azar. Basile (1914, 1914*a*) attempted to explain the negative results obtained by other workers by assuming that certain meteorological conditions existed during his experiments which were absent during those of other observers. It is of interest to note here that the writer (1912*c*), experimenting with fleas by Nöller's method, could obtain no evidence of the development of *L. tropica* in these insects (see below). Nicolle and Anderson (1923, 1924) have published an account of most careful attempts to transmit kala azar to ten dogs by means of numerous dog fleas which had fed upon known infected animals. The exposure to the fleas lasted from three weeks to seven months, but in no case did an infection result. Two of the dogs were made to swallow 510 and 410 fleas. Every source of fallacy was excluded, and it is rightly concluded that the experiments lend no support to the flea transmission hypothesis.

Mosquitoes.—Franchini (1911*a*, 1912) allowed *Anopheles maculipennis* to feed on cultures of *L. donovani*. The parasites persisted in the gut up to twenty-four hours, and leishmania forms were passed in the fæces. The same mosquitoes were fed on spleen puncture material from cases of kala azar. Leishmania were ingested, and persisted up to forty-eight hours. After thirty hours large round forms were present, while at the end of forty-eight hours a flagellate leptomonas form was found. He claims to have controlled his experiments by numerous dissections of mosquitoes not fed on leishmania material. Flagellate infections of *A. maculipennis* are, however, very common. No evidence of the possibility of transmission was produced. Patton (1907 and 1912*a*), in India, could obtain no evidence of any development of leishmania in *Culex pipiens*, *A. stephensi*, and *Stegomyia sugens*, but natural flagellates were found in some of these. Similarly, Mackie (1915) was completely unsuccessful with 266 culex and eighteen anopheles.

Lice.—Patton (1907–1912*a*) obtained negative results after feeding lice (*Pediculus capitis* and *P. vestimenti*) on kala azar cases in the blood of which leishmania occurred. Mackie (1915) fed large numbers of lice on kala azar patients, and, furthermore, dissected larger numbers collected from cases without finding any trace of leishmania in them. In all, over 3,000 lice were thus examined.

House Flies.—Patton noted that *L. donovani* degenerated very rapidly in the intestine of the house fly.

Ticks.—Patton, working with *Ornithodorus savignyi*, could obtain no evidence of development of *L. donovani*. Basile (1910*a*) and Marshall (1912) likewise had negative results with ticks.

Sand Flies.—The recent experiments of Knowles, Napier, and Smith (1925) have directed attention to sand flies of the genus *Phlebotomus*. They point out that Sinton has informed them that in India the distribution of *Phlebotomus argentipes* coincides with that of kala azar. An investigation of this fly in Calcutta has shown that twenty-five out of fifty-six female flies bred in the laboratory contracted a leptomonas infection after feeding on kala azar cases. Bred flies, forty-six in number, fed on control cases acquired no such infection, while 407 wild flies (317 ♀ and 90 ♂) also showed no infection. Similarly, 210 wild *P. minutus* were uninfected. Experimenting with *P. minutus*, it was found that this fly would not feed on man. One hundred

and three unidentified wild sand flies also gave a negative result, bringing the total of the controls to 857. Christophers, Shortt, and Barraud (1925, 1925*a*), and Shortt, Barraud and Craighead (1926), in Assam find the flagellates in massive numbers in the pharynx and extending to the buccal cavity. They state that this seems to be all that is required, short of a final proof of a transmission experiment, to demonstrate that kala azar is transmitted by the bite of the sand fly. With *Culicoides macrostoma* the first-named observers (1925*b*) obtained no development in forty-eight flies fed on cases.

ACTION OF DRUGS ON LEISHMANIA DONOVANI.—As regards the action of drugs on the parasites, a great advance was made by the introduction of tartar emetic treatment in kala azar by Di Cristina and Caronia (1913), after the success obtained by Vianna earlier in the same year with this remedy in dermal leishmaniasis of South America. Rogers (1915) introduced the treatment into India, and the drug is now widely used. It is recognized as a specific against the leishmania, and has so far influenced the mortality that, whereas formerly the majority of cases died, now the great majority recover.

The drug (or the corresponding sodium salt, which is supposed by some to be less toxic) is given intravenously. A dose of 10 c.c. of a 1 per cent. solution (*i.e.*, 1·5 grain) can be given once or twice a week during two to three months till a total of about 2 grams (30 grains) has been administered. Improvement, as shown by loss of fever, reduction in the size of the spleen, and a better general condition, takes place rapidly, but it is found that parasites still persist in the spleen, and are culturable even up to two months after the commencement of treatment, which must therefore be continued beyond this period.

Knowles (1920) found by culture that living leishmania might still be present in the spleen after 174 centigrams of tartar emetic had been administered intravenously. Stibacetin, or stibamine, first used in the treatment of kala azar by Caronia (1916), is an organic antimony compound which appears to be equally efficacious, but liable to a decomposition which renders it toxic. It can be administered intramuscularly. Urea stibamine, introduced by Bramachari (1922), stibamine glucoside and "von Heyden 471" have given good results, even in cases which were resistant to tartar emetic.

The Parasite of Oriental Sore.

Leishmania tropica (Wright, 1903).—This organism, which is morphologically indistinguishable from *L. donovani*, is the cause of the cutaneous infections known as oriental sore (Plate IV., p. 406). The disease occurs in the New as well as the Old World. In the latter, in the vast majority of cases the lesions are limited to the skin of the exposed part of the body, but as a very rare exception they may extend to the mucous lining of the mouth, nose, and pharynx. In the New World, though in most cases the lesions

are confined to the skin, they appear to be of a more chronic character than those of the Old World, while in about 10 per cent. of cases the mucosæ are involved. This latter condition produces in the mouth, pharynx, and nose extensive ulcerations and necroses, which may last for years, and reduce the victim to a condition of profound cachexia. In the Old World type, occasionally, ulceration may extend from the skin to the inner surface of the lips or nose, as recorded by Cardamatis and Melissidis (1911), in Greece by Pulvirenti (1913), and La Cava (1912 and 1914) in Italy, and by Christopherson (1914) in the Sudan. In these cases there is not the extensive involvement of the post-pharyngeal region so characteristic of the disease in South America. Castellani (1913), however, claims to have discovered leishmania in the pharyngeal ulcerations of a case in Ceylon. There had been no previous skin lesion, and, as Laveran (1917) remarks, if this was a case of infection with *L. tropica*, a fact which was not demonstrated, the case is quite abnormal and of an exceedingly rare type.

DISTRIBUTION.—The distribution of oriental sore is a very wide one. It occurs in Spain, Italy, and Greece, and more recently a case has been described from France by Ravaut (1920). It is fairly common in North Africa, and has been found in various localities along the northern coast area, especially at Biskra. It is found in Egypt and the Sudan, and also in the French Congo, in the district of Lake Chad, and on the Niger. Asia Minor, Arabia, Mesopotamia, Persia, and the southern parts of Russia are endemic centres. In India it is common along the north-west frontier districts, and even farther south, as at Cambay near Bombay. It does not, however, extend to the kala azar areas in the east. In America it occurs chiefly in Brazil and Peru, but also frequently in Guiana, Paraguay, Panama, Yucatan. Cases have also been recorded from the Argentine, Uruguay, Bolivia, Equador, Colombia, and Venezuela. The disease in the New World is known under various names, such as espundia, uta, buba, pian-bois, forest yaws, bosch yaws, and has been known for many years, though it was not till 1909 that Lindenberg, Carini, and Paranhos demonstrated leishmania in the ulcer of Bauru in Brazil. Splendore (1911) and Carini (1911) were the first to show that the disease of the naso-pharyngeal region was due to infection with leishmania.

It is thus quite clear that, though in most cases the disease in South America is limited to the skin, and in this respect resembles the disease of the Old World, in a certain percentage of the cases secondary lesions appear in the naso-pharyngeal mucosa and lead to a very chronic type of ulceration. Furthermore, the purely cutaneous type appears to be more severe and of longer duration than the oriental sore of the East, which rarely lasts for more than a year or eighteen months. It is possible, therefore, that the parasites causing the two diseases are not identical. Vianna

(1911) proposed the name *Leishmania brasiliensis* for the American form, on account of a filament he had observed in the parasites. This is undoubtedly the axoneme which is often demonstrable in *L. tropica* of the Old World. Escomel (1911, 1913*a*, 1914) noted elongate forms of the parasite provided with short flagella. He again (1922) refers to them, and gives a figure showing typical leptomonas. Accordingly, he proposed to name the organism *L. americana* var. *flagellata.* Rebagliati (1914) and Monge (1914) also claim to have observed flagellate forms of leishmania in the South American ulcers. La Cava (1912) has recorded similar forms in infections of *L. tropica* in Italy. Laveran and Nattan-Larrier (1912*a*) observed unusually large forms of the parasite in smears from a South American sore. There was a large central vacuole, and the nucleus was flattened out against one side of the parasite. On account of these peculiarities, they suggested the name *L. tropica* var. *americana* for the parasite. Exactly similar forms, however, are met with in the *L. tropica* of the East. Velez (1913), who discovered the disease in Peru, proposed to name the local parasite *L. peruviana.* It is evident that, of the various names proposed for the South American parasite, *L. brasiliensis* has priority over the others. No one, however, has been able to establish any morphological difference between this parasite and *L. tropica*, either as it occurs in the tissues or in cultures. The organisms cannot be distinguished from *L. tropica* except by the serological tests devised by Noguchi as described above (p. 399). Thomson and Balfour (1910) described a curious type of cutaneous leishmaniasis in the Sudan, in which the lesions were nodular and showed no tendency to ulceration. Here, again, the organism was morphologically indistinguishable from *L. tropica*, but Brumpt (1913*c*), regarding the disease as distinct from oriental sore, proposed to name the parasite *L. nilotica.*

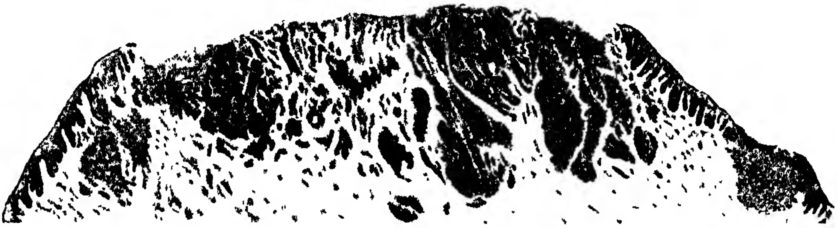
It seems better to retain the name *L. tropica* for both forms till more reliable proof of specific difference is forthcoming. On the other hand, the close resemblance of *L. tropica* to *L. donovani* led to Manson's suggestion that oriental sore is a local manifestation of an infection with the same organism that causes kala azar in much the same way as vaccinia may be supposed to be a local manifestation of smallpox. In this connection it is of interest to note that Nicolle and Manceaux (1910*a*) found that in experimental monkeys and dogs an animal recovered from oriental sore was immune to this disease but not to kala azar, while one recovered from kala azar was immune to both. Patton (1922) records an instance of a patient who contracted kala azar after having recovered from oriental sore. Laveran's experiments with mice show that they react differently to *L. tropica* and *L. donovani.* Furthermore, the distribution of the diseases is against the view of the identity of the two

organisms, though undoubtedly many arguments could be raised in support of their inclusion in a single species.

SYMPTOMOLOGY.—The cutaneous lesion due to *L. tropica* commences as a small, red papule, which is usually supposed to be the result of an insect bite. Instead of disappearing, however, it persists and increases in size, and may eventually give rise to a nodule an inch or more in diameter. After persisting for about a year, shrinking commences. The nodule finally dries into a scab, which eventually falls off, leaving a thin depressed scar. More usually, however, after a variable period of growth, the surface breaks down, and an ulcer with round edges is formed. Secondary bacterial infection takes place, and the ulcer may become as large as the palm of the hand. In the non-ulcerating variety, fluid obtained by puncture is found to contain large numbers of parasites, but in the ulcerating form these may be more difficult to detect, as scrapings from the granulating surface contain many pus cells and extraneous organisms. In such cases the best procedure to adopt in order to discover the parasites is to puncture the surrounding red margin of skin and run in a fine glass pipette, so as to obtain the tissue below the contaminated surface. Without the finding of the parasite, certain diagnosis is impossible, for the lesions often appear in remarkably atypical form, and even when they appear typical they resemble certain tropical ulcerations of quite another nature. If parasites cannot be demonstrated by direct examination, the culture method may be of assistance. In one case seen by the writer, an undiagnosed lesion on the ear contracted in South America had been treated unsuccessfully for several years. Though scrapings from the sore and puncture of the margin failed to reveal leishmania in stained films, yet flagellates grew in cultures inoculated with material obtained by puncture after sterilization of the skin. The organisms must have been very scanty, for it was not till after the lapse of three weeks that the characteristic organisms had multiplied sufficiently to be detected.

The lesions in oriental sore are usually confined to exposed surfaces of the body—*e.g.*, hands, wrists, feet, legs, and face. They are often single, but two or three sores are quite common. More rarely a larger number are present, and these may be scattered over the surface of the body. Cardamatis and Melissidis (1911) record a case in Greece in which there were thirty-five sores distributed about the hands, arms, and face, while Torres (1920) in South America observed one in which 248 distinct lesions occurred on various parts of the body. As a rule there is no constitutional disturbance, except in those South American cases in which naso-pharyngeal involvement occurs, when the patient is often very much reduced in health. Lymphangitis in the lymphatics and glands draining the infected region is not uncommon, and organisms have been obtained

by puncture of the enlarged glands. Neumann (1909*b*) on two occasions discovered leishmania in the peripheral blood of a case of oriental sore, an observation confirmed later by Patton (1912) in India. The presence of parasites in the peripheral blood in oriental sore is a very rare occurrence. The writer has searched in vain for them on many occasions, and attempts at culture from finger blood have given only negative results.



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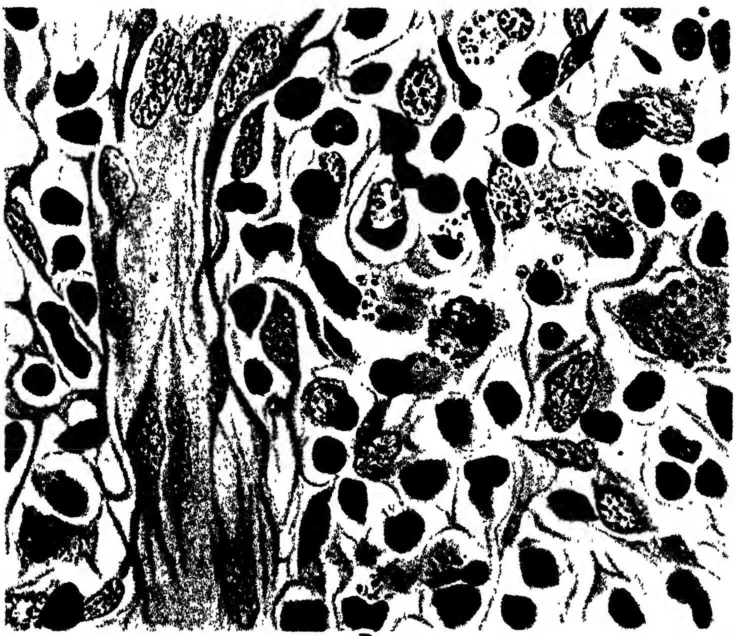


B

FIG. 191.—SECTIONS OF ORIENTAL SORE. (AFTER NATTAN-LARRIER, 1913.)

A. General view, showing elevated nature of sore, absence of epithelium on surface, and dark areas consisting of accumulations of macrophages (× 7).
B. Tissue of sore, showing macrophages containing *Leishmania tropica* (× 1,000).

PATHOLOGY.—In sections of oriental sore, especially the non-ulcerating variety, the new growth is found to consist of a fine reticulum of connective tissue, in the meshes of which are numbers of large cells often packed with parasites (Fig. 191). The cells resemble those met with in

cases of kala azar, and probably have a similar origin in the endothelial lining of the vessels. The epidermis over the new tissue is often very thin and degenerate, and in certain cases islets of epithelium are found more deeply. In the ulcerating form the deeper tissue is composed of large cells, but on the surface large numbers of pus cells are present also, and the structure resembles that of an ordinary granulation tissue.

MORPHOLOGY.—There is very little to add to the description given above for *L. donovani*, a parasite which cannot be distinguished morphologically from *L. tropica.* In any individual smear from a case of oriental sore, however, the parasites appear to have a greater range of form and size than they have in a smear from a case of kala-azar. The elongate, cigar-shaped forms are more frequently encountered in oriental sore, as also the larger types of parasite (Fig. 192). They occur in the cytoplasm of the large macrophages, and their appearance extracellularly in smears, as in the case of *L. donovani*, is due to rupture of cells in film-making. In the ulcerating variety of sore, where there has been secondary bacterial infection, and where pus cells are abundant, there is a tendency for degenerate parasites to appear, and in various stages of disintegration they may be found in these cells. In such cases it may be exceedingly difficult to identify them, and when yeast-like organisms are present also, confusion with these is easily made. Yeasts often stain in a manner closely resembling leishmania, and frequently exhibit a red area and a deeply-staining granule. Rocha Lima (1912) drew attention to this source of error, and pointed out that the organisms described by Darling in Panama as Protozoa under the name of *Histoplasma capsulatum* were in reality yeast-like bodies from a case of blastomycosis. Yeasts, however, have a much more distinct capsule, and the red area is more irregular in shape than the nucleus of leishmania, while the deeply-staining dot does not take on the characteristic rod-like form. Furthermore, reproduction takes place by budding, and evidence of this can generally be found. Another yeast-like organism, at one time considered to be a Protozoon

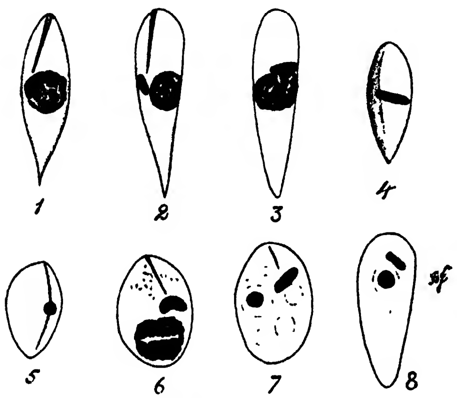


FIG. 192.—*Leishmania tropica* FROM ORIENTAL SORE (× *ca.* 5,000). (AFTER WENYON, 1911.)

1-3. Elongate torpedo- or cigar-shaped forms.
4-5. Abnormal forms in which kinetoplast alone is visible.
6. More rounded form with dividing nucleus.
7-8. Appearance after fixation with Schaudinn's fluid and staining with iron-hæmatoxylin.

allied to leishmania, is *Cryptococcus farcinimosus*, the cause of lymphangitis of horses (Plate III., p. 394).

The occurrence of flagellate forms of *Leishmania tropica* in oriental sore has been referred to above (p. 425). The writer has never seen such forms, though not infrequently fibres and filaments amongst the débris, especially if associated with parasites, may give the appearance of flagella.

CULTURE.—*Leishmania tropica*, which grows in artificial media as readily as *L. donovani*, was first successfully cultivated by Nicolle (1908*b*) in Tunis. Row (1909), in India, was also successful, as also were Markham Carter (1909), Marzinowsky (1909), and the writer (1911*a*). Pedroso (1910) appears to have first obtained culture of *L. tropica* from the South American disease. The writer (1912*b*) also cultivated the organism from a case of South American origin. The cultures of *L. tropica* behave like those of *L. donovani*, the most suitable temperature for growth varying from 22° to 25° C. It is possible that *L. tropica* grows more vigorously than *L. donovani*, but variation in individual strains is often considerable. In the writer's experience, certain strains of *L. tropica* grow very readily and others with greater difficulty, especially in the first subculture. Similarly, it sometimes appears that a slightly higher temperature is more favourable, but there is no uniformity in this. Giugni (1914*a*), for instance, claims that the optimum temperature for *L. tropica* is 28° to 29° C., while that for *L. donovani* is 21° to 22° C. The writer has kept many strains of both *L. tropica* and *L. donovani* growing for long periods at a temperature of 24°C. Nicolle (1925) reports having maintained a strain of *L. tropica* in culture for over fifteen years, during which it has passed through 384 sub-cultures.

Morphologically, the forms which appear in cultures of *L. tropica* are indistinguishable from those of *L. donovani* (Fig. 190). Some have maintained, as Row (1909) has done, that *L. tropica* produces larger forms than *L. donovani*, but the size of the flagellates varies with the age of the culture, and also with different batches of medium, which can never be prepared with absolutely uniform composition. Such a variety of forms occurs in the cultures, and these in such varying proportions, that comparison between different cultures is exceedingly difficult to make.

To obtain cultures from an oriental sore, it is necessary to secure material free from bacteria. In the ulcerating varieties, this can only be done by carefully sterilizing the skin at the edge of the ulcer with iodine or other antiseptic, making a puncture with a needle or sharp knife, and drawing off material by means of a sterile pipette. The material in the pipette is then blown into the liquid at the bottom of a tube of N.N.N. medium. Flagellates are to be detected in the tubes in from three days to three weeks, according to the number of organisms introduced.

As noted above (p. 399), Noguchi finds that the cultural forms of *L. tropica* can be distinguished from other species of *Leishmania* by serological tests.

NATURAL INFECTION OF ANIMALS.—Before the discovery of *L. tropica* in oriental sore, several observers had already noted that in localities in which the human disease occurred dogs were liable to develop similar ulcers, especially on the nose and ears. Neligan (1913), working in Teheran in Persia, where oriental sore is endemic, discovered leishmania in the cutaneous lesions of a dog. Not only were the parasites present in the skin lesions, but they were also found in the spleen, liver, and bone marrow. Yakimoff and Schokhor (1914) found leishmania in the cutaneous lesions of dogs in Turkestan, and they suggested the name *L. tropica* var. *canina* for this parasite. They produced no evidence, however, that it was different from the human parasite, which occurred in the same locality. Gachet (1915) examined twenty-one dogs in Teheran, and found skin lesions due to leishmania infection in fifteen of them. Dschunkowsky and Luhs (1909*b*), in Transcaucasia, discovered a dog with leishmania in the spleen, liver, and bone marrow. Avari and Mackie (1924) have discovered leishmania in ulcers on the ears of a dog in Bombay, and mention another similar infection of a dog in the Punjab which was brought to their notice by Row, who (1925) has described the case.

Thus, in Teheran and farther west in Transcaucasia, leishmania are found in dogs, not only in skin lesions, but also in the organs. The question arises as to whether here the two diseases, canine kala azar and canine oriental sore, exist side by side, or whether *L. tropica* in dogs leads to a general as well as a cutaneous infection. In the Mediterranean region. the naturally occurring canine kala azar is not associated with skin lesions, though a case of spontaneous cutaneous leishmaniasis of the dog has been noted by Sergent, Gueidon, Bouguet, and Catanei (1924) in Algeria, where canine kala azar occurs. In Transcaucasia, both oriental sore and kala azar exist in human beings, and it is not improbable that both occur in dogs. In Teheran, on the other hand, human kala azar is not known. It must be remembered that experimentally the virus of oriental sore may produce a general infection in inoculated animals, while that of kala azar can produce local skin lesions. The subject of canine leishmaniasis in these areas requires further investigation.

As regards the South American disease, Pedroso (1913) noted ulcers on the skin of two dogs which were associated with a man infected with *L. tropica.* In one of the ulcers the author claims to have found leishmania, but there seems to be some doubt as to the accuracy of this observation.

DIRECT INOCULATION FROM MAN TO MAN.—Before the discovery of *L. tropica*, it was well known that oriental sore could be handed on from man to man by inoculation of the skin with the material from a sore. In some places, such as Bagdad, Mosul, etc., it was the custom to inoculate on the arm or some covered part of the body, with a view to developing an immunity which would prevent the disfigurement of a natural infection on the face. One attack of the disease as a rule confers an immunity which lasts for the rest of life.

Definite evidence of the transference of the parasite in this way was first produced by Marzinowsky (1909), who inoculated himself. Parasites were demonstrated in the sore, which was first visible seventy days after inoculation. Nicolle and Manceaux (1910*a*) obtained a positive result by inoculation of cultures by scarification of the skin. The writer (1912*a*) inoculated himself in a similar manner with material from a sore in Aleppo. After a preliminary suppuration the wound healed, and it was not till nearly seven months later that a minute red speck appeared at the site of inoculation. This increased in size, and *L. tropica* was constantly present for one and a half years, during which it persisted.

Patton (1912) inoculated himself and developed a sore after sixteen days. Bouilliez (1917) inoculated himself accidentally. Material from a syringe entered the conjunctival sac, and about four months later a papule appeared on the internal surface of the lower lid. It increased in size to that of an almond, and a second papule appeared. *L. tropica* was demonstrated in the lesions. It is possible, therefore, for the parasite to infect a healthy mucous membrane. It does not appear to be able to pass the healthy skin, as was demonstrated by the writer. Material from a sore was placed on the healthy skin and allowed to dry naturally, but no sore developed at this spot, though at another spot where the skin was scarified a typical lesion resulted.

These experiments of direct inoculation have their parallels in natural infections. Numerous records occur of individuals who have developed oriental sore at the site of some accidental wound or abrasion of the skin. It is also well known that a person with one sore may auto-infect himself by scratching on other parts of the body.

SUSCEPTIBILITY OF ANIMALS.—It was first demonstrated by Nicolle and Manceaux (1910) that dogs could be inoculated in the skin with *L. tropica*, and that local cutaneous lesions containing the parasites resulted. Since then a number of observers have shown that dogs, cats, monkeys, rats, mice, and guinea-pigs can be similarly inoculated. In the case of small mammals such as mice, intraperitoneal inoculation has resulted in generalized infections, resembling in many respects those produced by the inoculation of *L. donovani*.

That the dog is susceptible to inoculation with *L. tropica* was first proved by Nicolle and Manceaux (1910). These animals developed sores after inoculation of virus from human cases or from cultures. The virus was handed on from dog to dog. Laveran (1915*d*, 1916) produced local lesions in dogs by inoculating material from the organs of mice which, as will be shown below, are liable to a generalized infection of *L. tropica.* Dogs which had recovered from a first infection are found to be reinoculable, but a second attack conferred an immunity against further infection. Attempts at the production of a general infection in dogs like that in kala azar, by injection of virus intraperitoneally or intravenously, by Nicolle and Manceaux, Laveran and the writer, have given only negative results. The duration of the inoculated disease in dogs is much shorter than in man.

With the South American virus the writer (1913) succeeded in inoculating a dog on the ear directly from a human case. A cat was also infected. Strong and his co-workers (1913) also infected a dog with the South American virus.

Monkeys were first inoculated by Nicolle and Sicre (1908*a*). Since then, Nicolle has extended his observations, and successful results have also been obtained by Row (1910), Patton (1912), Bouilliez (1917*a*), and Laveran (1912*d*, 1917). Various species of *Macacus* and *Cercopithecus* and the mandrill (*Cynocephalus mormon*) are found to be susceptible. The lesions produced resemble more closely those in man, but they are of shorter duration. With the South American virus the writer (1913) produced cutaneous lesions in a baboon. Sant' Anna (1913) successfully inoculated a species of *Cercopithecus.*

The observation made first by Row in India that local skin lesions could be produced in monkeys by inoculating *L. donovani* has been referred to above (p. 415).

Mice were first shown to be susceptible to *L. tropica* by Gonder (1913). The animals inoculated intraperitoneally with large doses of culture developed not only a general infection, but also swelling and cutaneous lesions of the legs and tail. Leishmania were present in all these lesions, and there was marked enlargement of the liver and spleen. General infections in mice were also produced by Row (1914*a*), Sergent, Ed. (1915), and Pavoni (1915), and especially by Laveran (1914*b*, 1915*b*, *c*), who has studied the question in detail. As a result of numerous experiments, it appears that in Laveran's hands the animals were easily infected by intraperitoneal injection of cultures or virus from other animals. In most cases, the first signs of infection in male mice, with which Laveran chiefly worked, is an infiltration of the peritesticular connective tissue, which becomes much thickened and œdematous, and is found to contain large numbers of parasites. Subsequent to this infection, a general infection of the internal organs takes place, associated with œdema of the limbs and tail. Fluid from these parts is found to contain parasites. The skin over the swollen testicular region, tail, and limbs breaks down and ulcers result. In female mice local skin lesions alone often appear.

Of a series of sixty-seven mice which were infected, forty-three showed only the local lesions without a general infection, fifteen had both local lesions and a slight general infection, while nine had local lesions and a fairly intense general infection. Mice as a rule do not show signs of infection for about a month.

The disease progresses for several months, and the animals may die or recover. The results of inoculation of mice with *L. tropica* thus appear to differ in a very striking manner from those obtained with *L. donovani.* Row (1914*a*, 1924), on the other hand, working in India, has produced general infections in mice with *L. tropica,* but has never noted the involvement of the skin or testes. The animals have reacted in every way as they do towards *L. donovani,* the parasites being numerous in the spleen, liver, and bone marrow. In some cases localized infections of the mucosa of the small intestine were noted in regions where lymphoid tissue occurred.

Laveran (1917) has shown that rats respond in a similar manner, especially if inoculated in the testicle. Bouilliez has also infected rats, and has noted local lesions in these animals. He was working in the district of Lake Chad (Chari River), and employed *Mus concha* and another rodent, which was probably *Arvicanthus niloticus richardi*. Another small rodent (*Golunda campanæ*) of this district was also infected by him. Laveran had similar results also with *Meriones shawi* and *Myoxus glis*. A guinea-pig inoculated in the testicle by Laveran with material from an infected mouse became locally infected, and a gerbil responded in the same manner as mice. Infection in animals has not always been a simple matter, for many observers have failed to produce infections, possibly because the dose of virus had been too small.

Franchini (1922*m*) states that he has infected the plant *Euphorbia segetalis* by inoculating it with cultures of *L. tropica*

TRANSMISSION.—At the present time it is generally believed that the sand flies of the genus *Phlebotomus* are responsible for the spread of oriental sore (Fig. 193). These flies were first suggested as possible vectors by Pressat (1905), and Sergent, Ed. and Et. (1905*a*), while the experiments conducted by Sergent, Ed. and Et., Parrot, Donatien, and Béguet (1921) in Algiers, by Aragão (1922) in South America, and Adler and Theodor (1925*a*) in Palestine, as also those of Laveran and Franchini (1920) in France, lend support to this view without, however, supplying the absolute proof. The last-named observers inoculated dogs in the skin with cultures of the leptomonas of *Phlebotomus*, and produced lesions resembling oriental sore, in which parasites were found. Similarly, Sergent and his co-workers (1921) produced a characteristic oriental sore in a man by inoculating crushed-up *Phlebotomus papatasi*, Aragão a similar sore on a dog by inoculating crushed-up *Phlebotomus* which had previously fed on a sore, and Adler and Theodor a papule containing leishmania on the skin of a man by inoculation of leptomonas from *P. papatasi*. It is possible that the leptomonas discovered by the writer (1911) in *Phlebotomus* of Aleppo was actually *Leishmania tropica*.

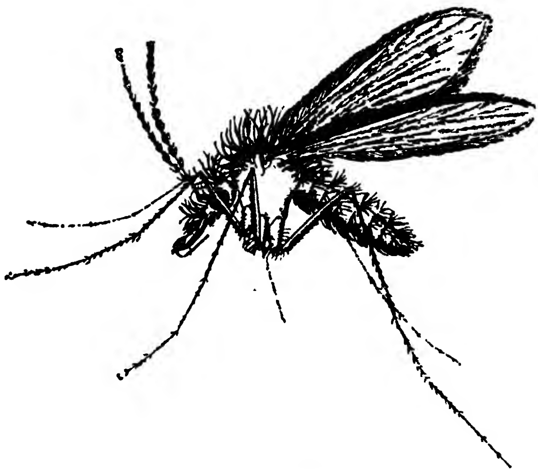


FIG. 193.—*Phlebotomus papatasi* (♀), THE PROBABLE TRANSMITTER OF *Leishmania tropica* (× *ca.* 13). (AFTER WHITTINGHAM AND ROOK, 1923.)

It was discovered by the Sergents, Lemaire, and Senevet (1914), and later by Chatton and Blanc (1918*b*), and Nicolle, Blanc, and Langeron

(1920), that the blood of the gecko (*Tarentola mauritanica*) harboured a flagellate of the leptomonas type, which was only demonstrable by culture of the heart blood. This gave rise to the view, first enunciated by the original discoverers of this organism, that the gecko, on which sand flies were known to feed, probably acted as a reservoir for the virus of oriental sore. Nicolle, Blanc, and Langeron (1920), by careful examination of the cultural forms of the gecko flagellate, concluded that they were distinguishable from the cultural forms of *L. tropica*. Moreover, injection of cultures of the gecko flagellate into the skin of man and monkeys failed to give rise to oriental sore. They conclude, with ample justification, that there is no real evidence that the gecko flagellate has any connection with *L. tropica*.

Strong (1924) has produced in the monkey a lesion resembling oriental sore in which leishmania occurred by subcutaneous inoculation of the skin with a flagellate of the leptomonas type, which occurs in the intestine of the lizard, *Cnemidophorus lemniscatus*, of Central America, where cutaneous leishmaniasis is endemic. The lizard, it is assumed, acquires its infection by feeding on plant bugs, which in their turn obtain the flagellates from the juices of Euphorbias (pp. 383, 442). Further investigations will be required before it can be accepted that the flagellate in this lesion is identical with that causing the naturally occurring human disease, or that the sequence of events described by Strong has any ætiological significance in connection with the natural method of its transmission.

It has been shown that *L. tropica* will develop in the bed bug like *L. donovani*, but there appears to be little reason for suspecting it to be a vector of oriental sore. Lloyd (1924) has found a typical *Leptomonas* in the proboscis and intestine of *Glossina morsitans* in Nigeria. As this fly feeds only on blood, it would appear that the flagellate must have been derived from the blood of some animal or man. As human leishmaniasis occurs in Nigeria, the flagellate of the tsetse fly may represent a *Leishmania*.

Experiments on the possibility of *L. tropica* developing in insects have been made by several observers.

Bugs.—The writer (1911*a*) observed that when the bed bug fed on an oriental sore before ulceration had set in, it took up leishmania, and a development similar to that described previously by Patton for *L. donovani*, took place. Patton (1912) published a more extensive series of experiments with bed bugs, and obtained results similar to those he had obtained with the parasite of kala azar. Working later in England, the writer (1912*c*) again found that *L. tropica* developed into flagellates in the bed bug. In no cases did active multiplication occur such as would be expected in the true invertebrate host, and, as with *L. donovani*, it appeared that the blood in the stomach of the bug had acted merely as a culture medium.

By a series of ingenious arguments similar to those employed in support of his claim of the transmission of kala azar by bed bugs, Patton attempted to prove that this insect also transmitted oriental sore. The bed bug was supposed to bite

exposed surfaces of the body more commonly than any other part. Whatever may be said in favour of the bed bug being a possible vector of *L. donovani*, no sound arguments, epidemiological or other, can be adduced in support of the claim that it is the cause of oriental sore.

Patton (1922) stated that he has been able to obtain a development of *L. tropica* in the bed bug similar to that obtained by Adie with *L. donovani* (see p. 419). Presumably, intracellular stages were seen, but as these occurred in the cells of the gut only after its removal from the body and incubation at a suitable temperature, they can hardly be recognized as representing a normal process of development, and still less as proving conclusively that the bed bug is the true host of *L. tropica* in Cambay in India, as Patton maintains. No host can be regarded as being conclusively incriminated in the transmission of *L. tropica* or any other parasite till the infection has been actually transmitted by it.

Fleas.—Working with fleas (*Pulex irritans* and *Ctenocephalus canis*), which the writer (1912*c*) fed on an oriental sore resulting from his inoculation in Aleppo, no evidence of development of *L. tropica* could be obtained. In these experiments the fleas were attached to wire according to Nöller's method, and before feeding on the sore were proved, by examination of the fæces ejected during feeding, to be free from flagellate infection. When fed on the sore, it was noted that leishmania were ejected with the fæces even in the first portion passed, proving that the fleas had actually ingested parasites. The fleas were then incubated at 22° C., the optimum temperature for culture. They were fed from time to time on the wrist, but no evidence of flagellates which might have developed from the leishmania could be found in the ejected fæces. Fleas found naturally infected with leptomonas constantly passed flagellates in the fæces. The fleas which had given negative results for leishmania were then fed on a rat harbouring *Trypanosoma lewisi*, and afterwards on the wrist as before. On the sixth day infective forms appeared, and continued in the fæces, thus proving that the conditions of the experiment were suitable for the development of a natural flagellate of fleas.

Laveran (1917) describes attempts to transmit *L. tropica* from mouse to mouse by means of fleas. Four healthy mice, together with others heavily infected with *L. tropica*, were placed in a glass jar which was serving as a flea breeding-place. There were so many fleas present that the mice had eventually to be removed for fear of their being killed by continued abstraction of blood. Three of the mice were examined after five months, and the fourth after eight months, but no infection had taken place.

Lice.—Patton could obtain no evidence of the development of *L. tropica* in lice.

Mosquitoes.—The writer (1911*a*), working in Bagdad, fed thirty-one *Culex fatigans* on oriental sore. It had been proved by dissection immediately after feeding that mosquitoes readily took up leishmania from a sore of the non-ulcerating variety. The mosquitoes dissected twenty-four, forty-eight, and seventy-two hours after feeding showed no trace of flagellates. In a few out of a large number of *Ædes argenteus* (*Stegomyia fasciata*) which had fed daily on the sore, rounded bodies possibly derived from the leishmania were found on dissection twenty-four or forty-eight hours after feeding. An attempt at transmission by means of twenty-six of these mosquitoes which had fed repeatedly on the sore and then on the arm gave no result.

Phlebotomus.—The writer (1911) first recorded the existence of a natural *Leptomonas* of the sand fly in Aleppo, an endemic centre of oriental sore. What is probably the same flagellate has been found in *P. papatasi* in Palestine by Adler and Theodor (1925*a*). It is possible that the flagellate was actually *Leishmania tropica*. Mackie (1914*b*) then gave the name *Herpetomonas phlebotomi* to a flagellate

found in *Phlebotomus minutus* in Assam. Shortt (1925) has examined the original preparations, and finds that it is actually a *Bodo*, the name of which is therefore *Bodo phlebotomi*.

Later Mackie again encountered flagellates in sand flies in the same locality. On this occasion elongate forms definitely crithidial in type were present in the films which were seen by the writer, so that it is evident that the flagellate was not a leptomonas. It may represent a trypanosome of a lizard, on which these flies are known to feed. Laveran and Franchini (1920, 1920*b*) state that they found a flagellate which they call *H. phlebotomi* in *P. papatasi* in Italy. In this case, again, the figures of the organism might be interpreted as representing crithidia. Cultures were obtained, and with them two dogs were inoculated in the skin of the thigh. One developed a local lesion resembling oriental sore, and the other a generalized infection like kala azar. In both cases leishmania forms of the flagellate were said to occur in the lesions. Patton (1919, 1920) refers to *H. phlebotomi* in connection with remarks on the probable transmission of oriental sore in Mesopotamia by *P. papatasi* and *P. minutus*. He has informed the writer that he did not actually see the flagellates in these flies.

Sergent, Ed. and Et., Parrot, Donatien, and Béguet (1921) had sand flies sent from Biskra to Algiers, a three days' journey. On one occasion seven *P. papatasi* received were crushed in saline and inoculated into the skin of a human being by scarification. Two months and twenty-four days later a papule which changed into a typical sore containing leishmania appeared, though flagellates had not been seen in the inoculated material. Aragão (1922) in South America fed *P. intermedius* on sores, and three days later crushed them in saline. This material was applied to a scarification on the nose of a dog, which developed a sore in which leishmania were found. These experiments prove that the sand fly can carry the virus in a virulent form for at least three days, for in the case of the flies employed by the French observers it is possible that they had just fed on a sore in the military hospital at Biskra, where they were caught. The experiments of Adler and Theodor (1925*a*) are more conclusive. In a single *P. papatasi* in Palestine numerous leptomonas were found in the whole extent of the alimentary canal, including the œsophagus and its diverticulum. The flagellates were inoculated into the skin of a human being on June 26. On July 31 a small papule had formed, and in it leishmania were found. Adler informs the writer that another positive inoculation from a naturally infected fly has been made, while flies have been infected with flagellates by allowing them to feed on oriental sores.

Hippoboscidæ.—Gachet (1915) noted that the dogs of Teheran were heavily infested with *Hippobosca canina*. Examining a fly which had just gorged itself on a sore on the face of a dog, leishmania were found in the blood in its stomach. Gachet thinks that the frequence of cutaneous leishmaniasis of dogs in Teheran may be due to the prevalence of this fly.

Stomoxys.—The writer (1911*a*) showed that Stomoxys were capable of taking up leishmania from a sore, but no development took place.

House Flies.—Laveran (1880*b*) first suggested that the oriental sore of Biskra might be due to fly transmission. The writer (1911*a*) and Patton (1912) experimented with house flies, but found that the leishmania degenerated after being ingested. Cardamatis and Melissidis (1911*a*) claim that *L. tropica* persists in flies up to six days, but they were undoubtedly observing the natural flagellates of the fly. It is, however, highly probable that the house fly, which swarms around the exposed sores, especially in children, may sometimes carry the virus on its feet or proboscis to abrasions on the skin of another person. The leishmania may also pass rapidly through the gut of the fly and be deposited with the dejecta, as occurs with other

organisms. Thus, trichomonas in fæces will appear quite unaltered in the dejecta of the fly five minutes after being taken up.

With reference to the cutaneous leishmaniasis of South America, there has been much speculation as to the transmitting host. Biting flies and ticks of various kinds have been blamed, but little definite observation has been carried out. Townsend (1915) inoculated a guinea-pig in the skin with flagellates he found in a Chironomid (*Forcipomyia*). A papule developed at the site of inoculation, and bodies supposed to be of the nature of leishmania were found in it. As the flagellates occur neither in the proboscis nor salivary glands of the fly, he believes that transmission is effected by deposition of fly dejecta in the skin, and subsequent contamination of the puncture wound inflicted. No proof was produced that the organism, if an organism at all, in the papule was in reality *L. tropica.* The experiments conducted by Aragão (1922), which have been noted above, suggest the possibility of *Phlebotomus intermedius* being the vector of the South American cutaneous leishmaniasis, while the observations of Strong (1924) suggest a possible connection with the flagellates of Euphorbias.

ACTION OF DRUGS ON LEISHMANIA TROPICA.—As in the case of *L. donovani*, tartar emetic and the corresponding sodium salt have a specific action on the parasites. Cures may be effected by scraping, excision, and the use of strong reagents, which not only destroy the parasites, but the tissues as well. Such are crystals of permanganate of potash, carbolic and nitric acids, solid carbon dioxide, and methylene blue. Tartar emetic may be used as in the case of kala azar, or in the form of an ointment locally. Emetin, as first pointed out by Photinos (1920), brings about death of the parasite and a cure of the disease when injected into the lesion.

Possibility of Confusing Leishmania with other Organisms.

Huntemüller (1914) described under the name of *Plasmosoma jerichœnse* an organism he had found in sections of tissue removed from a " Jericho boil." He considered it to be an entirely new Protozoon. The writer was able to examine the sections, which showed the organism to be badly-stained *Leishmania tropica*, which is often very difficult to stain in tissues, especially when unsuitably fixed. Similarly, Chalmers and Kamar (1920) described as *Toxoplasma pyrogenes* certain structures obtained from the spleen of a fatal case of splenomegaly in the Sudan. From information the writer has received, there is no doubt that the supposed toxoplasma was merely degenerating or badly-fixed *Leishmania.*

Similarly, the yeast-like organism *Cryptococcus farcinimosus*, which was discovered and named by Rivolta (1873), was regarded by many observers as a Protozoon, though its original discoverer had recognized its true nature. Rocha Lima (1912) drew attention to the fact that yeasts, as seen in stained smears, often simulated leishmania (Plate III., p. 394). Such a fallacy has always to be borne in mind when the organs of animals, especially those which have died and the tissues of which may have been invaded by bacteria or yeasts, are examined for *Leishmania.*

LEISHMANIA IN ANIMALS.

The definition of the genus *Leishmania*, which has been adopted here, is such that it includes all flagellates which attain the leptomonas form, and which have both a vertebrate and an invertebrate host. The latter feature is in the nature of an assumption, for, as shown above, the actual invertebrate hosts of *L. donovani* and *L. tropica* have not been demonstrated, though the probability of such hosts existing is so great as to amount almost to a certainty. In addition to the two forms already considered as producing diseases in man, there exist certain other leptomonas forms, which have been described as natural infections of vertebrates, and which must be included in this genus, though here also the invertebrate host has yet to be demonstrated (see p. 398). These naturally occurring infections are not to be compared with the artificial ones which Laveran and Franchini, and Fantham and Porter, claim to have produced in animals by the injection into them of purely insect flagellates. The latter have been considered in the section devoted to the insect flagellates (see p. 392). It seems probable that the naturally occurring infections in lizards result from their feeding on infected insects.

Leishmania tarentolæ Wenyon, 1921.—Sergent, Ed. and Et., Lemaire, and Senevet (1914), while searching for a host of *L. tropica* in North Africa, discovered that cultures of a typical leptomonas could be obtained from the heart blood and organs of the gecko, *Tarentola mauritanica*. The cultures closely resembled those of *L. tropica*, and led to the view that the gecko was a possible reservoir host of the human parasite, especially as the sand fly *Phlebotomus*, the supposed vector of oriental sore, frequently feeds on the lizard. The observation was confirmed by Chatton and Blanc (1918*b*), and by Nicolle, Blanc, and Langeron (1920) at Tamerza. The latter observers studied the cultures carefully, and came to the conclusion that the flagellates could be distinguished from those in cultures of *L. tropica*. The organisms must be present in the heart blood of the lizard in very small numbers, for in two positive cases out of twelve geckos examined, flagellates were not to be detected in the cultures till twenty-four to thirty-six days had elapsed. Nicolle and his co-workers believe that the organism is probably of intestinal origin, and is only accidentally present in the blood. Laveran (1915) could obtain no infection in geckos by inoculating them with *L. tropica*. Chatton and Blanc (1918*b*) inoculated geckos with cultures of *L. tarentolæ*, and were able to recover the flagellate from heart blood by culture in 50 per cent. of the cases after one to two months. In nature, 35 per cent. of geckos were found infected. Cultures of trypanosomes (*T. platydactyli*) were also obtained, but these could be readily distinguished. They also inoculated geckos with cultures

of *L. tropica*, and were able to recover the organism by cultures of heart blood even after the expiry of twelve days. Pittaluga and Buen (1917) in Spain, and Laveran and Franchini (1921*a*) in Italy, have examined specimens of *T. mauritanica* by the culture method, and have found them infected with *L. tarentolæ*. Laveran and Franchini state that the living flagellates were actually observed in the blood, but in most cases the presence of the organism was demonstrated by the culture method only. Cultures of the trypanosomes were also obtained. Franchini (1921*a*) states that a further examination of these lizards has shown that the flagellate may occur in the rectum and cloaca in the leptomonas and leishmania form.

By feeding bed bugs on geckos, Chatton and Blanc (1918*a*) obtained a temporary development of *L. tarentolæ* in the stomachs of the bugs.

Leishmania henrici (Leger, 1918).—This organism, discovered by M. Leger (1918*b*), and named by him *Leptomonas henrici*, was present in the blood of two out of thirty lizards (Genus *Anolis*) examined in Martinique. The body of the flagellate measured 15 to 16 microns in length and 4 to 5 microns in breadth. The flagellum was longer than the body. Leishmania forms were also seen, but more rarely. Leger subsequently found that over half the lizards harboured what was apparently the same organism in the rectum, so that there had probably been an invasion of the blood and organs from the intestine. The flagellate probably originates from some insect upon which the lizards feed, a fact which indicates how a leishmania infection may arise from an insect flagellate first becoming established in the intestine of the vertebrate. It opens up the possibility of *L. donovani* infecting man by way of the intestinal tract.

Leishmania chamæleonis Wenyon, 1921.—A typical leptomonas flagellate was observed by Bayon (1915) in the cloaca of *Chamæleon pumilus* of Robben Island. What was undoubtedly the same organism was discovered by the writer (1921) in Egypt in *C. vulgaris*. The flagellate was present in the cloaca in enormous numbers, where they lived in the mucus or invaded the lumen of the glands (Fig. 194). Intracellular forms were not seen, nor were cultures obtained from the heart blood. The measurements given by Bayon are incorrect, as the writer, who saw his preparations, can testify. The flagellate has a body about 15 microns in length, and the flagellum is slightly longer than this (Fig. 195). The width of the body in the long forms was about 3 microns. From these long flagellates may be traced a series of gradually diminishing individuals of varying size and shape, till minute round forms barely 2 microns in diameter are produced.

The last have relatively long flagella. Others are devoid of flagella and have the leishmania form, and some oval bodies with deeply staining

outline appeared to be encysted (Fig. 195, *d*). Some experiments conducted with house flies showed that a temporary infection of the gut resulted from feeding them on the cloacal mucus. It is in this material, rather than in the actual fæces that the flagellate occurs.

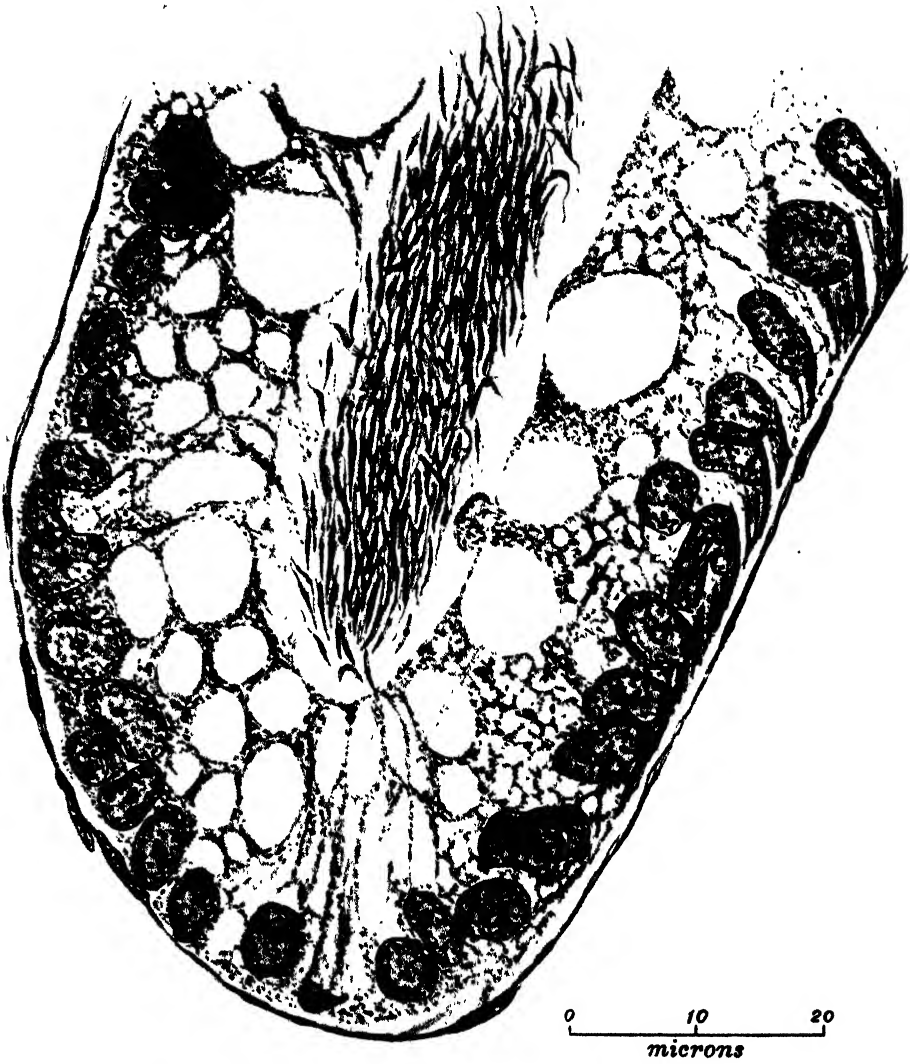


FIG. 194.—*Leishmania chamæleonis* IN THE LUMEN OF A GLAND OF THE CLOACA OF *Chamæleon vulgaris* (× 1,700). (AFTER WENYON, 1920; FROM *Parasitology*, vol. xii.)

Franchini (1921*a*) has examined two specimens of *C. vulgaris*, and has noted that the flagellates may occur in small numbers in the upper parts of the intestine. Leishmania forms are said to occur in the stomach.

No infection of the blood or other organs could be detected. Two mice which were fed on cloacal contents were said to have become infected. Free leishmania forms are described as occurring in the heart blood and bone marrow, and both these and leptomonas forms in the liver and spleen. By employing Nöller's blood-agar plate method, cultures of the flagellate were obtained from the cloacal contents of a chameleon. The flagellate of the chameleon is of interest when compared with *L. henrici*, which occurred, not only in the intestine of its host, but also in the blood. Further investigation of the flagellate of the chameleon will probably show that it also may occur in the blood-stream.

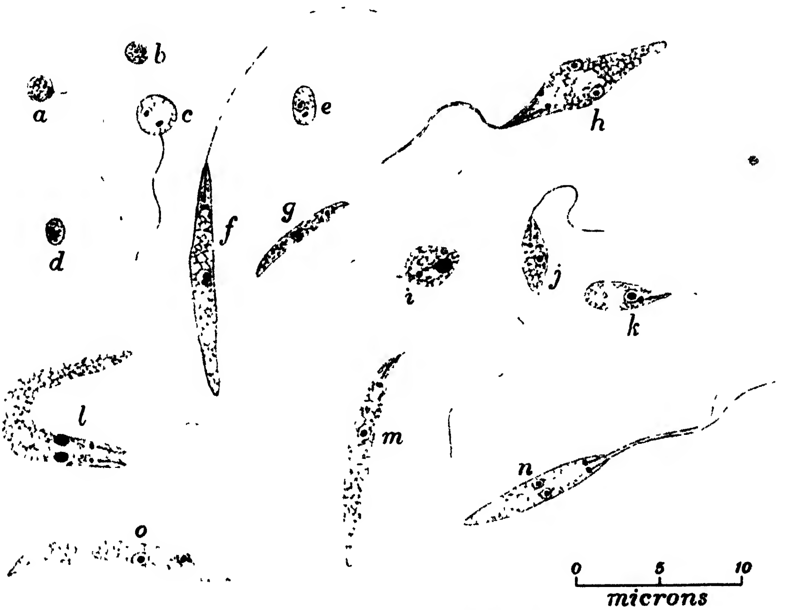


FIG. 195.—VARIOUS TYPES OF *Leishmania chamæleonis* FOUND IN CLOACA OF *Chamæleon vulgaris* (× 2,200). (AFTER WENYON, 1921; FROM *Parasitology*, vol xii.).

Leishmania hemidactyli (Mackie, Gupta, and Swaminath, 1923).—This parasite appears to be very similar to *L. tarentolæ*. It was discovered by the authors, who named it, in cultures made from the blood of the Indian gecko, *Hemidactylus gleadovii*. Direct examination of the blood failed to reveal any flagellates, and no mention is made of a concurrent intestinal infection. A trypanosome named *Trypanosoma hemidactyli* was also present in the blood.

Franchini (1921*a*) records the presence of leptomonas and leishmania forms of a flagellate in the rectum and cloaca, and also other parts of the gut, of *Lacerta ocellata*. They were also said to be present in the leishmania form in the heart blood and liver.

Another flagellate of the leptomonas type has been found by Strong (1924) in the hind-gut of the lizard, *Cnemidophorus lemniscatus.* He suggests that the infection is acquired by the lizards eating certain plant bugs which harbour what he assumes, on morphological grounds, to be the same organism. The bugs become infected by feeding on the latex of Euphorbias, which are also infected with the same flagellate. These observations, combined with the fact that Strong has succeeded in inoculating the lizard flagellate into the skin of the monkey, where a lesion resembling oriental sore is produced, serves to indicate the close relationship of all the flagellates of the leptomonas type.

Leishmania denticis (Fantham and Porter, 1919).—This flagellate was found in four out of forty-one silver fish (*Dentex argyrozona*) examined by Fantham (1919) and Fantham and Porter (1920) in South Africa. It was called by them *Herpetomonas denticis,* but is a flagellate of the typical leptomonas form, while leishmania stages also occur. The body measures 5 to 24 microns in length and 1·5 to 2·5 microns in breadth. The flagellum is often longer than the body, and is relatively longer in the shorter forms. Non-flagellate leishmania forms measured 2·5 to 4·5 microns by 1·5 to 2·5 microns. The organism was found most frequently in the heart blood, and also in smears of the liver, spleen, and kidney. It was not abundant in any fish examined, nor was it present in the intestinal tract.

Fantham (1922) records as *H. xenopi* a flagellate from the rectum of the South African clawed toad, *Xenopis lævis.* No details of the infection or of the flagellate are given.

Leishmania myoxi (Laveran and Franchini, 1921).—Three out of seven dormice (*Myoxus glis*) captured near Bologna were found infected. The organism, named *Herpetomonas myoxi,* was found only in stained smears of the blood, spleen, and liver. It occurred mostly as leishmania forms, which measured 1·8 to 3·6 microns in length by 1·2 microns in breadth. They were either free or within mononuclear cells. In addition, a certain number of elongate non-flagellate forms were seen. These measured from 5 to 20 microns in length by 1·2 microns in breadth. Flagellate leptomonas forms 12 to 20 microns in length were also encountered. Though the figures depict an organism of the leptomonas type, the writer feels that confirmation is necessary before the statements regarding it are accepted.

Genus: **Trypanosoma** Gruby, 1843.

The flagellates of the genus *Trypanosoma* attain the trypanosome structure at some stage of their development, and occur as parasites in the blood and tissues of vertebrate animals. For many of them there have been demonstrated invertebrate hosts, which transmit them from one vertebrate to another either by direct inoculation through the mouth

parts in the act of feeding, or indirectly by the vertebrate accidentally ingesting the infective fæces. The vast majority of trypanosomes are known only as they occur in the blood of the vertebrate, but it is safe to assume that an invertebrate host exists for every one, with the possible exception of *Trypanosoma equiperdum*, the cause of dourine, which is handed on directly from horse to horse during the sexual act. In some instances the trypanosome is only known in the invertebrate, but that a vertebrate host also exists is rendered probable by the fact that typical infections can be produced in laboratory animals by inoculating them with these insect flagellates. Such infections differ from the transitory infections which may result from the inoculation of purely insect leptomonas, crithidia, or herpetomonas, which, as explained above, cannot be regarded as having vertebrate hosts.

Trypanosomes have been found in every class of vertebrate, and it is because some of them produce disease in man and domestic animals that these flagellates have attained considerable importance and have been the subject of many investigations, the literature dealing with which is now very extensive.

According to Laveran and Mesnil, whose excellent treatise on trypanosomes and trypanosomiasis summarizes our knowledge of these flagellates up to the year 1912, the first observer to see a member of the genus was Valentin of Berne, who discovered a trypanosome in the blood of the trout, *Salmo fario*, in 1841. In the two succeeding years Gluge of Brussels, Mayer of Bonne, and Gruby of Paris published three papers on the trypanosomes of the frog. To these organisms Gruby gave the name *Trypanosoma*. From 1843 to 1880 little advance was made in our knowledge of trypanosomes except for their discovery in various amphibia, the black rat, the field mouse, and the mole. Timothy Lewis (1878, 1879) published accounts of the trypanosome of the rat in India, but these flagellates were first recognized as of great importance on the announcement in India of the discovery of a trypanosome in the blood of horses and camels suffering from surra by Griffith Evans (1880), and in the disease nagana of horses and cattle in Africa by Bruce (1895). Discovery of various other trypanosomes in domestic and other animals followed these observations, which led up to the discovery in the blood of a man in the Gambia by Forde of an organism which was recognized and described as a trypanosome (*T. gambiense*) by Dutton (1902). The next observer to see a trypanosome in man was Castellani, who (1903) announced his discovery of a trypanosome in the cerebro-spinal fluid of a case of sleeping sickness in Uganda. This observation was confirmed immediately afterwards by Bruce and Nabarro (1903), who demonstrated the causal relationship between the trypanosome and the disease.

Though it was long known that the diseases caused by trypanosomes in man and domestic animals in Africa were transmitted by flies belonging to the genus *Glossina*, the rôle of these blood-sucking diptera was not properly understood till Kleine (1909, 1909*a*) proved that a period of about twenty days was required for development of the trypanosome in the fly before the latter was able to bring about infection. Before Kleine's discovery in 1909, the repeated failure to transmit infections by tsetse flies had led observers to hold the view that they acted merely in a mechanical manner in carrying infective blood from one animal to another, with a lapse of a minimal interval of time between the two bites.

The work of Rabinowitsch and Kempner, Swingle, Nöller, the writer, Minchin and Thompson, and others established the rôle of the flea in transmitting *T. lewisi* from rat to rat, and proved that a development took place in the flea, leading to the appearance of infective forms of the trypanosome in the flea fæces which were eaten by other rats.

Brumpt, Robertson, Nöller, and others demonstrated the development of trypanosomes of fish and frogs in leeches, and the part they play in handing on the infection from one animal to another. The work of Chagas, Brumpt, and others has proved the rôle of *Triatoma megista* and other reduviid bugs in the transmission of the human trypanosome, *Trypanosoma cruzi*, of South America, while, finally, the observations of Nöller, Kleine, and Hoare have proved the transmission of *T. melophagium* of sheep by the sheep ked (*Melophagus ovinus*).

METHODS OF DISTINGUISHING TRYPANOSOMES.

The number of trypanosomes which have been named is very great, and the list is constantly being extended. It becomes of importance, therefore, to be able to distinguish one from another, and it has resulted that, quite apart from morphological details, various methods of separating the species have been devised. In many quarters, the discovery of a trypanosome in a new host has been taken as sufficient ground for the creation of a new species. Though this procedure is not in accordance with the rules of nomenclature, there is something to be said in favour of it, for the trypanosome of a particular host will be referred to by its name till it has been definitely proved to be identical with some other previously named type. This is likely to lead to less confusion in the literature than if workers had to deal with a large number of unnamed trypanosomes, or trypanosomes which had received already existing names because of certain resemblances they might have to these. Scientifically, it is just as incorrect to group together under one name without sufficient evidence what may eventually prove to be distinct species as to give different names to forms which ultimately may be found identical.

The characteristics which are of use in distinguishing trypanosomes are the following:

1. **MOVEMENT.**—When viewed alive under the cover-glass, trypanosomes vary very much in the movements they perform. Some are sluggish and do little more than wriggle and twist about in a limited area. This is true of many of the larger trypanosomes, like those of fish and frogs. On the other hand, amongst smaller types there is a similar variation. *T. gambiense* is moderately motile, and may travel some distance across the field of the microscope. *T. lewisi* is more active, while *T. vivax* takes its name from its remarkable motility. It darts about amongst the red blood-corpuscles and quickly passes out of the field.

2. **MORPHOLOGY.** — The morphological features of trypanosomes depend on the size and shape of the body, variations in the size and position of the nucleus and kinetoplast, and the degree of development of the undulating membrane and flagellum. All these features, as also others, have to be taken into consideration in describing the characters of any trypanosome. There may, however, be considerable difficulty in doing this, as they vary at different stages of development, and anything like a complete cycle is known only in a few instances. Thus, *T. lewisi* varies remarkably at different stages of development in the rat and the flea (Fig. 197). Similar variations occur in the case of *T. cruzi* of man (Figs. 207, 209), *T. rotatorium* of the frog (Fig. 237), and indeed, in all trypanosomes in which anything approaching a complete life-history is known. As in most cases only one stage in the development has been seen, and that in the blood of the vertebrate, knowledge of the exact morphology of these forms is very incomplete, and has often led to different stages of one and the same trypanosome being described as distinct species.

The general shape of the body of a trypanosome is that of a curved, flattened blade (Fig. 150). One margin of the body is generally convex and the other concave. The ends are tapering. The nucleus lies most usually near the centre of the body, and the kinetoplast near the posterior end. The axoneme commences at the blepharoplast, and after traversing the cytoplasm for a short distance passes along the border of the undulating membrane, which arises from the convex edge of the body as a thin ridge of cytoplasm. At the posterior end of the body, the membrane terminates and the axoneme may or may not be continued into a flagellum. Though on first appearance many trypanosomes seem to differ structurally from this type, they are, however, all traceable to it. The variations which occur may be considered as arising in one of two ways. Firstly, there may be an increase in the length of the convex border, giving rise to forms which are more and more curved till a complete spiral may be reached (Fig. 236, 2). Secondly, there may be a great increase in the width of the flagellate,

leading to forms which are remarkably broad (Fig. 150, 37). In some cases, both these modifications occur, with the result that there arise the very remarkable leaf-like trypanosomes which are seen particularly in amphibia (Fig. 150, 37).

In other cases increase in thickness as well as breadth occurs, and solid ovoid forms arise which are again typically seen in frogs (Fig. 150, 39, and 238).

It must be remembered that in ordinary stained films of blood these complicated forms are generally distorted to such an extent that their actual shape is obscured. The true form of the body can only be satisfactorily seen in the living condition or in specimens fixed without drying. Furthermore, during life the trypanosome is constantly altering its shape by contractions of its body, but in relaxation it returns to one or other of the types indicated in the diagram (Fig. 150).

The length of the body behind the kinetoplast is also subject to variation. In some forms the kinetoplast is actually at the posterior extremity, or very near it, as in *T. vivax*, *T. congolense*, and the metacyclic or infective forms of *T. lewisi* in the flea (Fig. 197, 20-23). In other trypanosomes this region of the body may be greatly prolonged, as in certain forms of *T. lewisi* in the rat (Fig. 197, 1-3) and the trypanosomes which occur in toads (Fig. 238).

The shape of the posterior end of the body is of some diagnostic importance, though it must not be forgotten that the extremity is subject to changes brought about by contractions of the living cytoplasm. Some trypanosomes, like *T. lewisi* and *T. cruzi*, have habitually a very sharply pointed posterior end, while others like *T. vivax* and *T. congolense*, have this extremity rounded (Plate V., p. 456). Many pathogenic trypanosomes not infrequently have the posterior end sharply cut off or flattened. The swollen condition of the posterior half of the body in *T. vivax* is highly characteristic of this species (Fig. 231).

The undulating membrane naturally has its attached border shorter than the free one, so that it is thrown into folds. The degree of undulation varies in different trypanosomes, and consequently the length of the axoneme. The degree of undulation is usually judged by the appearance of the axoneme. In *T. lewisi*, in the forms which occur late in the infection of a rat, the attached flagellum is only slightly undulating, whereas in *T. gambiense* it is much more so (Plate V. A and L, p. 456). In other trypanosomes the degree of undulation may be still more marked.

As already noted, the axoneme may terminate at the anterior extremity of the body, as in *T. congolense* and the stumpy forms of *T. brucei* and *T. gambiense*, or it may be extended as a flagellum (free flagellum) for a varying distance, as in the majority of forms (Plate V., p. 456).

So far reference has been made only to the flagellates of the trypanosome type, but it must be remembered that during the evolution of trypanosomes, either in the vertebrate or invertebrate host, other types appear—viz., crithidia, leptomonas, or leishmania forms. Thus, during the early stages of infection of the rat with *T. lewisi*, a great variety of forms may be found in the blood and organs, as also in its other host, the flea (Fig. 197). During the development of *T. gambiense* and other trypanosomes in tsetse flies, crithidia and other forms are found (Fig. 223). *T. cruzi*, though appearing in the blood of the vertebrate as small flagellates of the trypanosome type, reproduces intracellularly in the organs as leishmania forms, and presents a still greater variation in structure in its invertebrate host, the reduviid bug, *Triatoma megista* (Figs. 206, 207, 209). It will thus be apparent that, before accurate knowledge of the morphology of any trypanosome can be claimed, it is necessary to study every stage of its development, both in the vertebrate and invertebrate hosts.

The cytoplasm of a trypanosome is usually clear and homogeneous or finely alveolar, but there is frequently present a vacuole near the kinetoplast. Sometimes the cytoplasm contains granules which are greenish and refractile in life, and stain deeply purple with Romanowsky stains. They are most frequently seen in the anterior region of the body, and probably consist of volutin. According to Doflein (1916), the cytoplasm of cultural forms of *T. rotatorium* of frogs may contain droplets of a fatty substance. In some of the larger trypanosomes longitudinal markings of the surface of the body have been described, and these are generally regarded as contractile fibres or myonemes lying in the outer layer of the cytoplasm (Fig. 28, B). There is no definite ectoplasm layer as distinct from an endoplasm, but the surface of the body is limited by fine membrane or periplast representing a concentration of the superficial cytoplasm. The undulating membrane may be regarded as a lateral extension of this limiting layer of denser cytoplasm.

The nucleus is typically spherical, and consists of a nuclear membrane enclosing a clear material at the centre of which lies a karyosome (Fig. 156). It is situated usually at the centre of the flagellate. In some cases, as in the posterior nuclear forms of *T. brucei*, it may lie near the posterior end of the body and sometimes actually behind the kinetoplast (Fig. 224). In others, as for instance, in *T. lewisi*, it has moved in the reverse direction, and is typically found anterior to the central point of the body (Fig. 197, 19).

The kinetoplast, consisting of the blepharoplast and parabasal, as explained above (p. 329), lies at a short distance from the posterior end of the body. It may actually be situated at the extreme posterior end, as in

T. congolense. The parabasal part of the kinetoplast varies considerably. It is a comparatively large body, often slightly elongated, or egg-shaped in *T. cruzi.* It is smaller and spherical in most pathogenic forms, while in *T. equinum* of the South American disease of horses (mal de Caderas), it is apparently absent (Plate V., 1, p. 456).

In a single blood-film from an infected animal it will be found that the trypanosomes are not all of the same size, and in the case of some pathogenic forms (*T. gambiense*, *T. brucei*) it has been the custom to describe those present as belonging either to the "short stumpy," "intermediate," or "long thin" forms (Figs. 221, 224). The measurement of the trypanosomes and the relative positions of the various structures may be roughly made by the micrometer eye-piece, but the flagellates are often

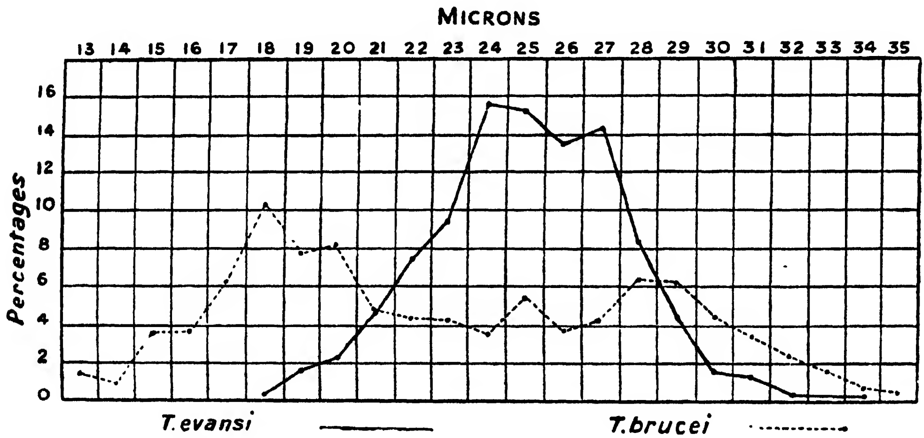


FIG. 196.—CURVES REPRESENTING THE DISTRIBUTION, BY PERCENTAGES, IN RESPECT TO LENGTH OF *Trypanosoma evansi* AND *Trypanosoma brucei*. (AFTER BRUCE, 1911.)

so twisted in the stained film that this is a matter of difficulty. A more accurate method is to draw the trypanosomes to a fairly large scale with the aid of a camera lucida, and to project on to the paper the micrometer scale through the same system of lenses. The trypanosomes may then be measured by setting the measuring dividers according to the scale on the paper. A large number of individuals can thus be measured, and the average dimensions taken, while a curve can be plotted showing the percentage of trypanosomes of any length between the maximum and minimum (Fig. 196). A feature of these curves is that very frequently they show a notch which has been interpreted as an indication that in such infections two particular types of trypanosomes are present—not necessarily two species, but that the one species tends to produce in its develop-

ment two main groups of organisms. Some have suggested that it indicates a tendency towards the production of male and female individuals, but, at present, there is no evidence to support this view.

3. **PATHOGENICITY.**—Trypanosomes are often grouped as pathogenic and non-pathogenic forms. The former are those which give rise to disease in man or domestic animals, and they do so, not because these are their natural hosts, but because the man or animal is susceptible to inoculation with a trypanosome, which in its natural host is non-pathogenic. As a general statement, it is safe to regard all trypanosomes as non-pathogenic to their natural hosts. The vast majority resemble *T. lewisi* of the rat, which, under ordinary circumstances, cannot infect any other host than the rat. A small number of trypanosomes are, however, inoculable into man, the domestic animals, and experimental laboratory animals, and in these unnatural hosts they often produce serious symptoms of disease. To their natural hosts, which in many cases are the big game of Africa, they are apparently harmless. The various pathogenic trypanosomes, however, vary in the effect they produce on laboratory animals, and these variations are of some assistance in the identification of the species. *T. brucei*, for instance, is readily inoculated into the rat, mouse, and guinea-pig, whereas, with *T. pecorum*, these animals cannot be infected. The human strain of *T. brucei* (*T. rhodesiense*) inoculated from man into the rat rapidly produces a very heavy infection which quickly kills the animal, whereas *T. gambiense* under these circumstances may fail to infect the rat, though it usually does so, leading to a chronic type of infection characterized by the presence of a small number of trypanosomes in the blood at any time, the animals surviving for even a year or more. By passage from rat to rat the virulence of such a strain may be increased, till finally the infection may become as intense and as rapidly fatal as that produced by *T. brucei*.

The animals most frequently employed for these tests are rats, mice, guinea-pigs, dogs, monkeys, goats, and even the larger domestic animals, such as donkeys, horses, mules, and cattle.

It sometimes happens that in the inoculated animals there appear particular forms of trypanosome which were not present in the original host. Thus, in man, *T. brucei* (*T. rhodesiense*) closely resembles *T. gambiense*, but in the inoculated rat there appear a certain number of posterior-nuclear forms which enable the trypanosomes to be distinguished from *T. gambiense* (Plate V. A and B, p. 456).

In these experiments it is of importance to note the period of incubation before trypanosomes appear in the blood of the inoculated animals, the intensity of the infection produced, and the duration of the infection.

In some animals, particularly the goat, only transitory infections are produced, the animals quickly ridding themselves of their trypanosomes. The incubation period and the subsequent course of the infection varies with the dose or number of trypanosomes injected, and also with the type of injection, the intraperitoneal route leading to more certain and rapid infection than the subcutaneous. Naturally, any conditions which lower the vitality of the experimental animals at the same time lower their resistance, and lead to a more intense infection.

4. **IMMUNITY.**—As noted above, some animals, though acquiring an infection from inoculation, recover after a lapse of time. This is particularly true of the goat, sheep, and ox. Such recovered animals are found to be resistant to reinoculation with the same trypanosome, but are still susceptible to another. Laveran and Mesnil have employed this method extensively in differentiating trypanosomes. Thus, a goat rendered immune to *T. brucei* (*T. rhodesiense*), as proved by reinoculations, was susceptible to *T. gambiense*. Though this method will undoubtedly distinguish trypanosomes of distinct species, it is possible that mere races of one and the same species may give similar results. Furthermore, animals which have recovered from an infection with one species may sometimes be reinfected with the same species. Thus, Nöller (1913*b*) has shown that frogs may be infected twice or even three times with the trypanosome, which occurs naturally in these animals (*T. rotatorium*). Such superimposed infections demonstrate that in many cases the immunity acquired against any particular trypanosome is very inconstant, and that great caution has to be exercised in making deductions from such cross-infection experiments. Martin and Darré (1912) gave an account of a trypanosome which had been acquired accidentally by Lanfranchi when working with *T. evansi* in the laboratory. The strain of trypanosome recovered from his blood, and also that with which he supposed he had infected himself, were investigated by Mesnil and Blanchard (1914). Lanfranchi stated it was *T. evansi*, but Mesnil and Blanchard, by use of immunity tests, concluded that the two strains were different. They were, however, unable to identify the human strain with any known trypanosome, and decided to refer to it as "*Trypanosoma Lanfranchi.*" Such a result is a direct indication of the unreliability of the immunity test. In his work on *T. melophagium* of sheep referred to below, Hoare (1923) has shown that so long as sheep are infested with keds, trypanosomes are present in the blood. If the keds are removed, the infection disappears in two or three months, only to reappear again when exposure to keds again takes place. In this case it would seem that any batch of trypanosomes introduced by a ked are able to multiply in the sheep and survive for a limited period. If their disappearance is due to an acquired

immunity on the part of the sheep, this at any rate is insufficient to prevent a fresh infection.

5. **SEROLOGY.**—Rabinowitsch and Kempner (1899) were the first to demonstrate the protective property of the serum of animals recovered from trypanosome infections. They showed that 0·5 c.c. of serum from a rat recovered from an infection due to *T. lewisi* was sufficient to protect a normal rat against infection when the serum and blood containing trypanosomes were inoculated at the same time. Laveran and Mesnil (1901*a*) extended this observation, and demonstrated that if sufficient serum from an animal, such as the sheep or goat, rendered immune to any particular trypanosome was mixed with infective blood from another animal containing the same trypanosome, normal animals inoculated with the mixture did not become infected, whereas the same serum mixed with another trypanosome did not protect against infection with the latter. This property of the serum for destroying trypanosomes and preventing infection may be retained for long periods. The serum of a chronic case of sleeping sickness in man has a similar action in the case of *T. gambiense*, whereas the serum of a normal individual is not protective. Furthermore, Laveran (1902*b*, 1903) was the first to demonstrate that normal human serum had a marked protective action when inoculated to mice at the same time as either *T. brucei*, *T. evansi*, or *T. equinum*. The normal human serum was in some cases even curative when injected into animals already infected.

Mesnil and Ringenbach (1911) showed that it had a similar action on the human strain of *T. brucei* (*T. rhodesiense*). Laveran and Nattan-Larrier (1912*c*) discovered that this reaction was far from constant, as different human strains of this trypanosome behaved differently towards human serum. Freshly isolated strains tended to be killed by human serum, while this ceased to be the case after the strain had been subjected to many passages through laboratory animals. Laveran (1915*a*) showed that one particular strain of *T. gambiense*, even after being kept for twelve years in laboratory animals, still resisted normal human serum. Mesnil and Blanchard (1916), however, proved that other strains of *T. gambiense* may lose this resistance after long periods. Conversely, *T. brucei* may acquire a resistance not previously possessed by it after many passages, as proved by Jacoby (1909). Laveran (1904*a*) also demonstrated that the serum of the higher apes, especially the baboons (*Cynocephalus*), behaved like human serum. Mice injected with serum at the same time as *T. brucei* did not become infected, whereas, with *T. gambiense*, they were not protected.

The serum of animals recovered from infections may have a trypanolytic or disintegrating action on the trypanosomes *in vitro*, as first shown

by Franke (1905) in the case of the serum of cattle recovered from *T. brucei* infections. Laveran and Mesnil (1900*a*, 1901*a*) first demonstrated the agglutinating action of the serum of recovered rats on *T. lewisi*. When the serum is allowed to act upon the trypanosomes *in vitro*, they become arranged in clusters or rosettes with their flagellar ends directed outwards (Fig. 152, A). Though the trypanosomes are attached to one another by their posterior ends, there is no loss of activity, as evidenced by the continued movement of the flagella. After some time the cluster breaks up, and the individual trypanosomes swim away. The phenomenon is often termed "agglomeration" to distinguish it from bacterial agglutination, which involves loss of vitality of the individual bacteria, there being no tendency for the clumps to break up.

Laveran and Mesnil (1901*a*) also found that if *T. lewisi* were injected into the peritoneal cavity of rats which had recovered from an infection, the trypanosomes quickly became attached to leucocytes, while no such attachment occurred in the case of rats not previously infected. This observation was extended to other trypanosomes by Mesnil and Brimont (1908, 1909), while Levaditi and Mutermilch (1910) studied it in detail. The last observers found that the reaction depended on immune substances in the blood of recovered animals, for the addition of immune serum from these recovered animals to a mixture of trypanosomes and cells obtained from artificially produced peritoneal exudate caused the trypanosomes to become attached to the cells. The serum still retained this power even after heating. This reaction, though in certain cases quite specific, is too inconstant to be relied upon as a means of differentiating trypanosomes.

Many experiments have been made to test the deviation or fixation of complement in trypanosome infections with a view to diagnosis, but the results so far obtained are very discordant. Sometimes, however, definite and uniform results are obtained, as in the work of Watson (1915), and Woods and Morris (1918) on infections of horses with *T. equiperdum*. Woods and Morris, working with dogs, found that, using as antigen a salt solution of the spleen of a heavily infected animal, complement fixation usually followed, but sometimes occurred before, the appearance of trypanosomes in the blood of the dog. The reaction, however, always appeared before clinical manifestations of disease. Therapeutic injections of arsenobenzol into infected dogs not only caused the trypanosomes and clinical symptoms to disappear, but so altered the serum that the complement fixation test became negative, as in normal dogs.

The complement fixation test has been employed on a large scale for diagnostic purposes in the case of dourine of horses in Canada. The

technique devised by Watson (1915 and 1920) was used. As antigen, trypanosomes from heavily infected rat's blood were employed. They were separated by repeated centrifugation in saline solutions from the blood-cells and sera.

The tests were carried out as in the Wassermann test for syphilis. Between the years 1912 and 1919 it was applied to 40,000 horses in Canada, with the result that infection was detected in many animals which clinically were not suspected of suffering from dourine. By adopting the practice of slaughtering all animals giving a positive reaction, the disease has not only been prevented from spreading, but has been almost, if not entirely, stamped out. Writing of these results, Watson (1920) points out that the test is absolutely reliable from a diagnostic point of view. Clinically, the incubation period of the disease may vary from two weeks to three months, but from the results obtained by the serological test it became apparent that the animals show signs of infection in from ten to twenty days.

Schoening (1924), using the dourine antigen, applied the test to camels to be imported into the United States, and was able to demonstrate that a trypanosome infection was present. The organism in these animals was probably *T. evansi*, so that the positive result obtained proved that the test is not specific for any particular species of trypanosome. It is evidently what is termed a group reaction.

Very interesting serological studies with cultures of frog trypanosomes (*T. rotatorium*) have been made by Nöller (1917). He employed the flagellates grown on horse blood-agar plates, so that the cultural forms could be removed with a minimal amount of admixture with the ingredients of the culture medium. Horse serum produced sedimentation in emulsions of the flagellates in a dilution of 1 in 20, and a macroscopic agglutination in 1 in 40 to 1 in 80. The flagellates were all killed by the undiluted serum in one hour, while in a dilution of 1 in 10 the majority were killed in this time, and none were alive on the following day. With horse serum inactivated by heating to 56° C., sedimentation alone was obtained, and this only when the undiluted serum was used, whereas the agglutination up to 1 in 80 occurred with the active serum. The inactivated serum, moreover, had no trypanocidal action. Guinea-pig serum produced sedimentation in dilutions of 1 in 10 to 1 in 20, but appeared to have little agglutinating power. Its trypanocidal action, however, was marked, but ceased at a dilution of about 1 in 160. As Nöller remarks, this result is directly the opposite of that obtained by Mendeleeff-Goldberg (1913) in experiments conducted with cultures in the liquid of N.N.N. medium. The serum of infected frogs (*Rana esculenta*) gave sedimentation in dilution of 1 in 80 and agglutination in 1 in 40. The undiluted

serum killed most of the flagellates in a short time, a percentage of 1 or 2 surviving, whereas in dilutions of 1 in 10 only about 90 per cent. were killed. With higher dilutions the number of surviving flagellates increased. The inactivated serum (56° C.) had no trypanocidal action. The agglutinin was also destroyed, a result which shows it to be thermolabile, and thus different from the agglutinin which occurs in horse serum. Working with serum of uninfected frogs, similar results were obtained. It thus appears that the agglutinating action of the serum is no indication of the power of the animal to resist infection, for not only were uninfected frogs infected by injection of cultures, but superimposed fatal infections were produced in already infected frogs. These results appear to be analogous to those obtained by Mesnil and Blanchard (1916), who proved that human sera had a marked trypanocidal action on both *T. gambiense* and *T. brucei* (*T. rhodesiense*), both of which may infect human beings.

Though serological tests may serve to distinguish strains of trypanosomes, it does not follow that the trypanosomes thus differentiated are true species in the zoological sense. As in the case of the immunity test referred to above, different races of one and the same species may show differences in serological reaction. Ponselle (1923*a*) has produced a certain degree of immunity in mice with a vaccine of *T. brucei*. A solution consisting of dihydrogen potassium phosphate (H_2KPO_4) 1·8 grams, hydrogen disodium phosphate ($HNa_2PO_4,2H_2O$) 0·2 grams, and distilled water 100 c.c. is prepared and sterilized at 115° C. for twenty minutes. To 2·5 c.c. of this solution is added with sterile precautions 0·5 c.c. of heart blood of a mouse at the end stage of its infection with *T. brucei*. After twenty-four hours at 20° C. the mixture is inoculated intraperitoneally to mice in a dose of 0·1 c.c. It was found that in four or five days the serum of the mice had acquired definite agglutinating properties against *T. brucei*, and that in many cases after eight to ten days the mice were immune to inoculations with doses of trypanosomes which produced the usual rapid and fatal infections in control animals.

6. **CULTURE.**—The culture method, though it has been mostly employed to determine infections which are not evident on microscopical examination of the blood, as in the case of *T. theileri* of cattle and *T. melophagium* of sheep, has also been used for purposes of identification. Thus, Nöller, working with cultures of *Crithidia subulata* of *Tabanus glaucopis*, claims to have proved that this is merely the insect phase of *Trypanosoma theileri* on account of the exact similarity of the culture forms of each. He also demonstrated the similarity between cultures of the sheep trypanosome and the flagellate of the sheep ked (*Melophagus*

ovinus), and came to the conclusion that they were merely stages of one organism (*T. melophagium*), a fact which has been conclusively demonstrated by Hoare (1923).

The course of development of trypanosomes in cultures is of considerable interest, for, undoubtedly, it is an imitation of the development which takes place normally in the invertebrate host. Most observers have noted that the trypanosomes introduced into culture media commenced multiplying and became transformed into flagellates of the crithidia type. Thomson, J.D. (1908), first noted in the case of cultures of the trypanosome of gold fish that the first division process of the trypanosomes resulted in the formation of crithidia forms (Fig. 247). Active multiplication of these takes place for some time, but eventually trypanosome forms again appear. There seems little reason to doubt that these represent the metacyclic trypanosomes which appear at the end of the development in the leech. Delanoë (1911) noted that in old cultures of *T. lewisi* small trypanosomes appear, and it is evident from his figures that these correspond with the metacyclic trypanosomes which are developed in the rectum of fleas, and which are the actual infective forms. Hoare (1922, 1923), working with cultures of *T. melophagium*, both from the blood of sheep and from the intestine of the ked, has noted the same fact. Small trypanosomes appear in the cultures after the crithidial phase, and these are identical with the metacyclic trypanosomes which are developed naturally in the hind-gut of the ked. It seems clear, therefore, that the course of development of any trypanosome in culture is a parallel of the natural development in the invertebrate. Nöller (1920*c*), working with cultures of *T. loxiæ* and *T. syrnii* of birds, *T. theileri* of cattle, and *T. melophagium* of sheep on blood-agar plates, states that at low temperatures the flagellates remain in the crithidia form, but that elevation of the temperature to 37° C. causes their transformation into trypanosomes. It seems that the only possible biological explanation of this phenomenon is that the heightened temperature causes the flagellates to revert to the warm-blooded vertebrate phase, which is a step in advance of the metacyclic trypanosomes which appear in the cultures in liquid media at low temperatures. It would seem reasonable to suppose that the transformation noted by Nöller as occurring on agar plates after an elevation of temperature is not comparable with the appearance of trypanosomes in liquid media kept at a uniformly low temperature, but rather with the changes undergone by the metacyclic trypanosomes after they gain entrance to a vertebrate.

7. **INSECT VECTOR.**—Finally, the capacity to develop in invertebrate hosts may be employed as a means of differentiating trypanosomes. *T. gambiense* is capable of infecting *Glossina palpalis*, and only rarely

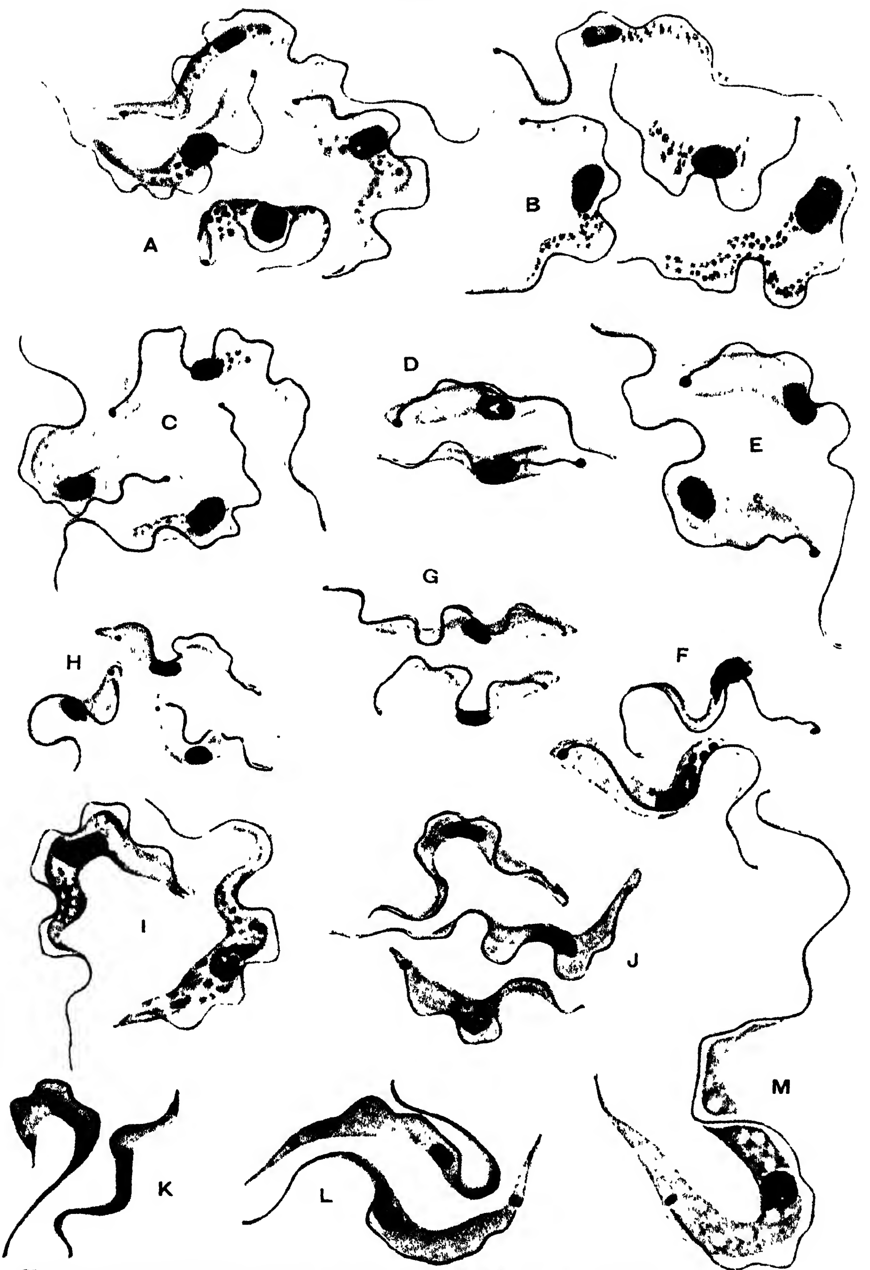
G. morsitans, whereas the reverse is the case with *T. brucei* (*T. rhodesiense*), though in the blood of man the two trypanosomes resemble one another closely. The power to infect invertebrates is not always as specific as this, for *T. lewisi* can undergo its development, not only in the rat fleas, but also in the dog and human fleas. This method of diagnosis has been named by Brumpt (1914*a*) *xenodiagnosis*. He found during his investigations into the development of trypanosomes of fish, frogs, and snakes that leeches often developed infections after feeding on animals in which no trypanosome had been found. Similarly, with *T. cruzi* various species of reduviid bugs may acquire infections when the trypanosomes are too scanty to be found by microscopical examination in the animals on which they fed. Bruce *et al.* (1913*a*, 1914*d*), in Nyasaland, employed the test in a reverse manner by feeding batches of tsetse flies on susceptible animals in order to determine the nature of the infection of the flies, the developmental forms of the trypanosomes in the flies being more difficult to identify than those in the blood of a vertebrate.

CLASSIFICATION OF TRYPANOSOMES.

At the present time our knowledge of the life-histories of the majority of described trypanosomes is so imperfect that it is impossible to classify them accurately in any system. Attempts have been made to divide the group into separate genera. For instance, Lühe (1906) proposed to separate the mammalian trypanosomes from all others under the generic name of *Trypanozoon*. More recently, Chalmers (1918) has attempted a still more elaborate classification, with the establishment of a number of genera which are quite indeterminate. Such attempts fail to assist in the clear understanding of this already complicated group, and only lead to greater confusion. On morphological grounds alone all the trypanosomes undoubtedly belong to one genus, *Trypanosoma*. Where anything like a complete history is known, they are found to be polymorphic, exhibiting in their development every type between the leishmania and the trypanosome form. As a rule, reproduction by fission of any of these forms may occur. Variations in the shape and size of the body, the relative positions of the kinetoplast and nucleus, the degree of development of the undulating membrane and flagellum, cannot be regarded as generic characters.

From the descriptions which will be given below it will be seen that there are two main courses of development in the invertebrate. There is the development which leads to infection of the biting parts of the invertebrate, so that the vertebrate is inoculated during the biting act; and, secondly, there is the development which leads to a hind-gut phase,

PLATE V.



Various trypanosomes of man and animals (x 2000).—A. *T. gambiense.* B. *T. brucei* (*T. rhodesiense*). C. *T. evansi.* D. *T. uniforme.* E. *T. caprae.* F. *T. vivax.* G. *T. simiae.* H. *T. congolense.* I. *T. equinum.* J. *T. equiperdum.* K. *T. cruzi.* L. *T. lewisi.* M. *T. theileri.* (After various authors and originals.)

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the vertebrate being infected by accidentally ingesting the fæces of the invertebrate or the invertebrate itself. In the former case the trypanosomes are described by Duke (1913) as having an *anterior station* in the invertebrate. Employing this suggestion, it will be convenient to describe the others as having a *posterior station.* In the case of *T. gambiense* in *Glossina palpalis,* infection of the salivary glands follows an intestinal phase of development (Fig. 216). In other trypanosomes (*T. congolense*), also transmitted by species of *Glossina,* an intestinal phase of development leads to infection of the proboscis alone, the salivary glands not being infected (Fig. 217), while in others (*T. vivax*) there is no intestinal development, the whole cycle taking place in the proboscis of the tsetse fly (Fig. 218). These three variations of development in the tsetse flies may be used as a basis for classifying some of the pathogenic trypanosomes, as has been done by Duke (1913) and Bruce (1914). Many of the trypanosomes of cold-blooded vertebrates, as, for instance, *T. inopinatum* of frogs, are transmitted by leeches. In these invertebrates the trypanosomes develop in the stomach, and finally in the proboscis sheath (anterior station), whence they gain access to the wound inflicted by the leech when it feeds (Fig. 243). All these methods of infection by tsetse flies and leeches may be spoken of as *inoculative,* since the trypanosomes are inoculated at the time of biting. The other method of infection is *contaminative,* for infection takes place through infected fæces or the invertebrate itself being ingested or, possibly in some cases, by fæcal contamination of the wound inflicted by the invertebrate. Thus *T. lewisi* develops in the flea, leading to infection of the rectum (Fig. 199). Rats acquire the infection by eating the fæces of the fleas. If the complete life-histories of all the trypanosomes were known, it might be possible to group them according to such data as have been just outlined. In the case of several trypanosomes of small rodents, it is now known that the invertebrate hosts are fleas, and that the infection of the vertebrate is contaminative as in *T. lewisi,* while the trypanosomes of fish are carried by leeches, as in the case of *T. inopinatum* of the frog. In a certain number of cases, however, the trypanosome is known only in its invertebrate host, but the existence of a vertebrate host is rendered highly probable from the fact that these flagellates are easily inoculable into vertebrates and produce a definite infection comparable with the infections produced by inoculation of trypanosomes from vertebrate to vertebrate. Thus, Lafont (1912) discovered a flagellate in the gut of *Conorhinus rubrofasciatus.* When inoculated to mice it produced a typical trypanosome infection, and for this reason he gave it the name *Trypanosoma boylei.*

According to the scheme given on p. 346, the trypanosomes can be grouped in the following manner:

Group A.—Trypanosomes which develop in the posterior station in the invertebrate:

I. Trypanosomes of rodents, Cheiroptera, Insectivora, Edentata, monkeys.

II. The trypanosome of man in South America, *T. cruzi*.

III. Non-pathogenic trypanosomes transmitted by species of *Tabanus*, *Melophagus*, or other blood-sucking Arthropoda, including the large forms from cattle, sheep, and antelopes.

Group B.—Trypanosomes which develop in the anterior station in the invertebrate or have become secondarily adapted to direct passage from vertebrate to vertebrate:

I. Pathogenic trypanosomes transmitted by blood-sucking Arthropoda.

II. Pathogenic trypanosomes secondarily adapted to direct passage from vertebrate to vertebrate.

III. Trypanosomes of birds (?).

IV. Trypanosomes of land reptiles (?).

V. Trypanosomes of aquatic vertebrates transmitted by leeches:

1. Trypanosomes of aquatic reptiles.
2. Trypanosomes of amphibia.
3. Trypanosomes of fish.

The pathogenic forms are those which produce disease in man and domestic animals, but these cannot be regarded as the natural hosts. In Africa, the pathogenic forms are naturally parasitic in the wild game, where they are relatively non-pathogenic. They only become pathogenic when inoculated into susceptible animals which have not developed a relative immunity as a result of exposure for many generations. The virulence of *T. lewisi*, which under natural conditions is quite harmless, may be increased till it becomes definitely pathogenic, and *T. inopinatum*, harmless for the African frogs, is pathogenic for those of France. The pathogenic trypanosomes, however, form a convenient group, and are transmitted in most cases by species of *Glossina* in Africa. There are some pathogenic trypanosomes, however, in the transmission of which the tsetse fly can play no part, as, for instance, *T. evansi* of surra and *T. equinum* of mal de Caderas, which occur in countries where tsetse flies are not found. In these cases other biting flies of the genus *Tabanus* and its allies fulfil the rôle. As regards *Trypanosoma equiperdum*, its affinities are undoubtedly with the trypanosomes of the pathogenic group. It appears that its capacity of passing directly from vertebrate to vertebrate has been secondarily acquired as a result of the situation of its

development in the vertebrate. It may be a form of *T. evansi* modified by long passage from vertebrate to vertebrate without an arthropod intermediary.

CURATIVE ACTION OF DRUGS AND SERA IN TRYPANOSOMIASIS.

The serum of certain normal animals when injected into rats or other laboratory animals infected with pathogenic trypanosomes will sometimes cause their temporary disappearance. For instance, a dose of 0·1 to 1 c.c. of human serum injected into a mouse infected with *T. brucei* may cause the trypanosomes to disappear entirely from the blood. Some strains of *T. brucei* resist such treatment, as also does the human strain (*T. rhodesiense*). The serum from animals, such as the goat, which have recovered from an infection is little more active than a normal serum, so that at present there seems little possibility of a serum therapy in trypanosomiasis being devised.

Much more definite results have been obtained with chemical agents. Ehrlich and Shiga (1904) gave an account of the action of the organic dye trypanrot on trypanosomes. They showed that a fair proportion of experimentally infected animals could be permanently cured by its means. A long series of investigations on allied organic compounds was carried out by Nicolle and Mesnil (1906), and it was found that a definite relationship existed between the structure of the molecule and the therapeutic action.

Thomas (1905) announced the fact that the organic arsenic compound atoxyl had a specific action on trypanosomes, and was very much less toxic than arsenious acid, which had previously been employed in the treatment of sleeping sickness. The introduction of atoxyl led to a series of investigations under the direction of Ehrlich, which resulted in the elucidation of the chemical nature of atoxyl and the preparation of other organic arsenic compounds, notably arsenophenylglycine, and finally salvarsan. Many other allied drugs were produced, and it is chiefly in one or other of these forms that arsenic is now employed in the treatment of trypanosomiasis.

Antimony in the form of sodium or potassium antimony tartarate (tartar emetic) has a marked action on trypanosomes, which disappear rapidly from the blood of animals after intravenous injection. As they disappear the trypanosomes show evident signs of degeneration, while smears from the spleen show quantities of débris from the broken-down organisms. Though the trypanosomes may disappear entirely from the blood after a single injection, they almost invariably reappear after a number of days. It seems apparent that it is rarely possible to give at a

single injection a dose sufficiently large to kill all the parasites and yet not to kill the host. Accordingly, in treating trypanosomiasis it is necessary to continue the treatment with small doses over long periods in the hope of ultimately killing all the trypanosomes or assisting the body to do so.

There is a danger in prolonged treatment that drug-fast strains may be created. It was noted that after treatment by a single dose of a drug trypanosomes reappeared after varying intervals. Further treatment caused them to disappear again. Eventually, after several relapses, the drug frequently became incapable of causing the organisms to disappear from the blood. This phenomenon was studied by Ehrlich and his co-workers. It was discovered that there was a real resistance on the part of the trypanosomes, for it persisted even when the trypanosomes had been subjected to many passages through animals which had had no previous injections of drugs. Strains resistant to various arsenic and antimony compounds were obtained. It was further demonstrated that certain arsenic-free substances, as, for instance, pyronine and acridine, were able to produce strains resistant to atoxyl. In some cases arsenic resistant strains could be made resistant to tartar emetic by injecting other arsenic compounds. These facts are of great importance from the point of view of treatment of trypanosome diseases. It may be that in this process a kind of natural selection occurs, the more resistant survivors always producing larger numbers of resistant forms after the susceptible ones have been killed by the drug. Mesnil and Brimont (1908*b*) have shown, however, that a race which had become resistant to atoxyl, and had maintained this resistance when passed through mice, lost it when transferred to the rat, only to regain it when again passed into the mouse. It is evident that the tissues of the host play a part in the therapeutic process.

A curious action of certain drugs, such as pyronine and others of the oxazin series, on trypanosomes was noticed first by Werbitzki (1910). If animals infected with *T. brucei* are treated with these drugs, it will be found that an increasing number of the trypanosomes lose the parabasal body in the kinetoplast. In some cases the strain becomes normal again after several passages through animals, but occasionally all the trypanosomes present show this peculiarity, which persists through many passages. The exact meaning of this alteration is not understood, but it is interesting to note that in *T. equinum* of horses of South America the parabasal is normally absent.

Voegtlin *et al.* (1920) have studied the action of various arsenic and antimony compounds on trypanosome infections. They note that the trivalent arsenic and antimony are markedly toxic for animals and also for trypanosomes, which disappear very rapidly after intravenous injections. The substances have a marked trypanocidal action *in vitro.* On the other

hand, the pentavalent arsenic and antimony compounds are much less toxic, do not cause the trypanosomes to disappear at once, and have no trypanocidal action. This indicates that if the arsenic or antimony compounds are in the form of R.As=O, symptoms of toxicity appear at once and trypanosomes disappear rapidly. If they are in the form of

$$R.As\begin{smallmatrix}\diagup OH\\ =O\\ \diagdown OH\end{smallmatrix}$$

a much longer time is required. It is concluded that during the interval or latent period the pentavalent compounds are being reduced in the body to trivalent ones. In the case of the arsenobenzol derivatives (salvarsan, etc.), which act slowly and have no trypanocidal action *in vitro*, it is believed that an oxidation to the trivalent forms takes place (R.As=As.R becomes R.As=O). In the case of atoxyl, which again shows a latent period before it acts, and which has no trypanocidal action *in vitro*, the process seems to be one of reduction to the trivalent form. Terry (1915) showed that if atoxyl and blood were incubated together, the mixture acquired marked trypanocidal properties.

In animals such as rats and mice the various salvarsan and neo-salvarsan compounds vary in their toxicity and in their therapeutic efficiency. The toxic dose for these animals varies from about 0·2 to 0·6 gram per kilogram of body weight, while a dose which is approximately one-tenth of this will clear the blood of pathogenic trypanosomes in about twenty-four hours. The toxic dose of tartar emetic is about 0·04 gram per kilogram of body weight, and a dose of 0·02 gram per kilogram will clear the blood of trypanosomes in about fifteen to thirty minutes.

A drug (Bayer 205), which was first introduced in Germany by Haendel and Joetten (1920), and Mayer and Zeiss (1920), appears to have an action on trypanosomes which is more specific than that of any drug hitherto employed. It is claimed that cures can be uniformly brought about in small animals, and also in horses suffering from dourine. Furthermore, in small animals the single dose (0·003 gram per kilogram of body weight) necessary to bring about a cure is only one-sixtieth of that which can with safety be given to the animals. The ratio between the minimal therapeutic dose and the maximum tolerated dose is thus 1:60. In this respect, again, the drug is superior to any trypanocide which has been used before. The writer (1921*b*) tried the drug in the case of mice infected with a very virulent strain of *T. equiperdum*, and was able to confirm the statement of the German investigators.

Kleine and Fischer (1922) and Kleine (1924) find that the drug is efficacious in the case of human trypanosomiasis, and also gives promising therapeutic and prophylactic results in the disease of domestic animals.

Low and Manson-Bahr (1923) have also obtained apparent cures in a large percentage of human cases treated by them. It appears that the drug gives a fair promise of cure only in the cases which have no involvement of the central nervous system. As regards the action of the drug on trypanosomiasis of domestic animals, Kleine and Fischer (1923) find that its action is less marked than in the case of human beings, while in the animals *T. brucei* is more responsive than *T. vivax* or the closely allied *T. capræ*. It appears that the more nearly the infected host resembles the natural reservoir, the less active is the drug. Thus, *T. brucei* is more readily eradicated from man than from cattle, for the latter are more closely related to the buffalo, which is one of the natural reservoirs of this trypanosome.

Another drug which has a marked trypanocidal action in the case of experimentally infected laboratory animals is tryparsamide, the sodium salt of N. phenylglycineamide-*p*-arsonic acid. Its action in sleeping sickness has been the subject of an investigation by Pearce (1921) in the Belgian Congo. Van den Branden and Van Hoof (1923) have followed up some of the cases treated by Pearce, and report that a cure can be effected in 100 per cent. of early cases of human trypanosomiasis in the Belgian Congo when the cerebro-spinal fluid is still normal, and that in a large percentage of more advanced cases a similarly successful result can be obtained.

In the treatment of human beings suffering from trypanosomiasis, the drugs hitherto most usually employed are atoxyl or soamin and tartar emetic. Injections of one or both of these must be continued over long periods, and cure may be effected in a certain number of cases. It must be remembered, however, that some cases tend towards a natural recovery, and appear to respond very well to treatment, while others get progressively worse in spite of the remedies used. On this account, great caution has to be exercised in ascribing good results to any particular line of treatment, while a cure cannot be said to have certainly taken place unless there have been no signs of the disease for some years.

In the treatment of trypanosomiasis of domestic animals, the above-mentioned compounds, as well as liquor arsenicalis, have been tried with varying success. Tartar emetic administered intravenously seems to give the best results. Hornby (1919), working in Rhodesia, noted that horses and other equidæ were more liable to infection with *T. brucei* than with *T. congolense* and *T. vivax*, while the reverse was the case for cattle. Hornby found that tartar emetic had little effect in saving horses infected with *T. brucei*, but was of great value for cattle harbouring *T. congolense* or *T. vivax*. He has informed the writer that as many as 80 per cent. of the cattle may be saved by the use of this drug if treatment is commenced

early. His practice is to give with a syringe 1 gram of the drug intravenously every five days till six doses have been injected. Though the animals may not be entirely cleared of infection, they are saved from death, improve clinically, and get into good condition again. If relapse or reinfection occurs, the treatment is repeated.

SYSTEMATIC DESCRIPTION OF SPECIES.

Group A. Trypanosomes which Develop in the Posterior Station in the Invertebrate.

I. TRYPANOSOMES OF RODENTS, CHEIROPTERA, INSECTIVORA, EDENTATA, CARNIVORA, AND MONKEYS.

(*a*) Trypanosomes of Rodents.

The best-known trypanosome of this group, *Trypanosoma lewisi* of the rat, will be considered as a representative of the group.

Trypanosoma lewisi (Kent, 1880).—Synonyms: *Herpetomonas lewisi* Kent, 1880; *Trypanomonas lewisi* (Labbé, 1881); *Trypanosoma rattorum* Börner, 1881; *Trichomonas lewisi* (Crookshank, 1886); *Trypanosoma sanguinis* Kanthak, Durham, and Blandford, 1898; *Trypanomonas murium* Danilewski, 1889; *Trypanozoon lewisi* (Lühe, 1906); *Trypanosoma longocaudense* Lingard, 1906.

According to Laveran and Mesnil (1912), the first person to see this trypanosome was Chaussat, who discovered it in the blood of *Rattus rattus*. He mistook it for a nematode embryo, and it was Lewis in 1877 who recognized as a flagellate the organism he saw in the blood of *R. decumanus* and *R. rufescens* in India. In his manual on Infusoria, Kent (1880) referred it to the genus *Herpetomonas*, as did also Bütschli (1884). Laveran and Mesnil (1901*d*) showed that this flagellate did not differ in any essential respect from the type of the genus *Trypanosoma* created by Gruby (1843) for the parasite of the frog, and that therefore the parasite of the blood of rats should be known as *T. lewisi*, which name it has retained, though several observers have needlessly attempted to create new genera for its reception.

Distribution.—*T. lewisi* is very common in *R. rattus* and *R. decumanus* in all parts of the world where these rats occur. In India it is found in *R. rufescens* and *R. niveiventer*, in Africa in *R. maurus*, in Christmas Island in *R. macleari*, in Tunis in *R. alexandrinus*. It has been recorded from other small rodents, though in many cases it is probable that the trypanosomes were not *T. lewisi*. A trypanosome of the S. African gerbil (*Tatera lobengula*) is regarded by Fantham (1925) as a race of *T. lewisi*.

Course of Infection in the Rat.—*T. lewisi*, which is readily inoculated from rat to rat, can be conveniently studied in the white rat. The try-

panosomes appear in the peripheral blood from four to six days after intraperitoneal inoculation of infected blood from another rat, and the resulting infection may be divided into two phases. In the first, a great variety of forms occurs in the blood, most of which are in process of division (Fig. 197). This is the multiplication phase, but it gradually subsides, giving place to a phase in which the trypanosomes are much more uniform in character, and are the forms generally recognized as *T. lewisi*. The first phase is of short duration, and multiplying forms are rarely seen in the peripheral blood after the eighth or ninth day, when the only forms to be found are those of the second phase, which lasts from one to four months. When inoculation has been made intraperitoneally—and this is the readiest method of bringing about infection—it is stated by Laveran and Mesnil (1912) that multiplication first commences in the peritoneal cavity, and that these stages are much more numerous in the peritoneal exudate than in the blood. Before their appearance in the peripheral blood after intraperitoneal inoculation, it appears, from still unpublished observations by A. C. Stevenson, that active multiplication has been taking place in the small vessels of the internal organs, especially the kidneys. He was unable to demonstrate the active multiplication in the peritoneal cavity, though in the later stages of an infection trypanosomes occurred in the exudate. Within two days of peritoneal inoculation, multiplying forms can be demonstrated in sections of the organs. In the ordinary course of events, *T. lewisi* does not seriously injure the rat, which recovers from its infection and nearly always has an immunity to reinfection. Miss M. Robertson, however, informs the writer that if only a slight infection occurs after a first inoculation, the rats may be reinfected. In some cases, rats are reported to have died as a result of heavy infections.

Roudsky (1910–1911), by rapid passage from rat to rat of the whole blood of an animal when the trypanosomes were at the multiplication phase, was able to raise the virulence of *T. lewisi* till it became definitely pathogenic to rats, and not only infected mice, which are seldom susceptible to the ordinary strains, but sometimes killed them. Further, the infection in mice was transmissible from mouse to mouse. This strain of heightened virulence was also inoculable to rabbits, guinea-pigs, and other rodents, which are rarely susceptible or entirely resistant to *T. lewisi*. It was suggested by Reichenow (1917) that the numerous trypanosomes of mice and other rodents, which morphologically resemble *T. lewisi*, and even a trypanosome which he found in African apes, might actually be *T. lewisi*. Yamasaki (1924) attempted without success to infect mice and monkeys by means of fleas which had become infective after feeding on rats.

Morphology.—The trypanosome form which is present in the blood of the rat for the longest time is the one which occurs after the multiplication phase (Fig. 197, 18–19, and Plate V., L, p. 456), and is generally spoken of as *T. lewisi*, though this name applies to all stages of its development in the rat and flea. The trypanosome as seen in the later stages of an infection is a very characteristic organism. Very similar forms occur in the blood of other small mammals, and they are often referred to as being of the *T. lewisi* type. These forms (Plate V., L, p. 456) are about 25 microns in length, and have a distinctly curved body which is sharply pointed at its posterior end. There is a well-developed kinetoplast situated at some distance from the pointed posterior extremity. The nucleus is definitely anterior to the central point of the body. The undulating membrane is not markedly convoluted, the axoneme along its border running a fairly straight course. There is a well-developed flagellum beyond the anterior extremity of the organism. The curved body, the sharp posterior end, and the excentric position of the nucleus give these forms of *T. lewisi* a very characteristic appearance. Apart from the nucleus and kinetoplast, the cytoplasm of the trypanosome is usually free from granules, but certain structures not always visible have been described as of occasional occurrence (see p. 323).

During the multiplication phase of *T. lewisi*, which commences shortly after inoculation, the trypanosomes which occur in the blood-vessels exhibit an extreme degree of polymorphism. There are large broad trypanosomes with prolonged and pointed posterior ends with their kinetoplasts adjacent to the nucleus, very much smaller forms of the same type, and small round forms provided with flagella. Types intermediate between all these also occur. These variations are best comprehended by reference to the figure (Fig. 197, 1–15).

In the living condition the typical trypanosomes are exceedingly active, and dash about with great energy amongst the red blood-corpuscles with flagellar end in front, quickly passing from one microscopic field to another. The large multiplication forms and others seen in the early stages of an infection are much less motile.

Multiplication.—As already noted, the multiplication phase is of short duration, and is characterized by the marked polymorphism of the trypanosomes. It may be said to commence with the large broad trypanosomes, which measure at least 35 microns in length and have the kinetoplast near the nucleus (Fig. 197, 1–3). Division of the kinetoplast takes place, followed by that of the nucleus. From the daughter kinetoplast is formed a new axoneme, which does not grow to the length of the original one, so that a short undulating membrane is formed. The cytoplasm then divides between the flagella, and a small daughter individual is separated.

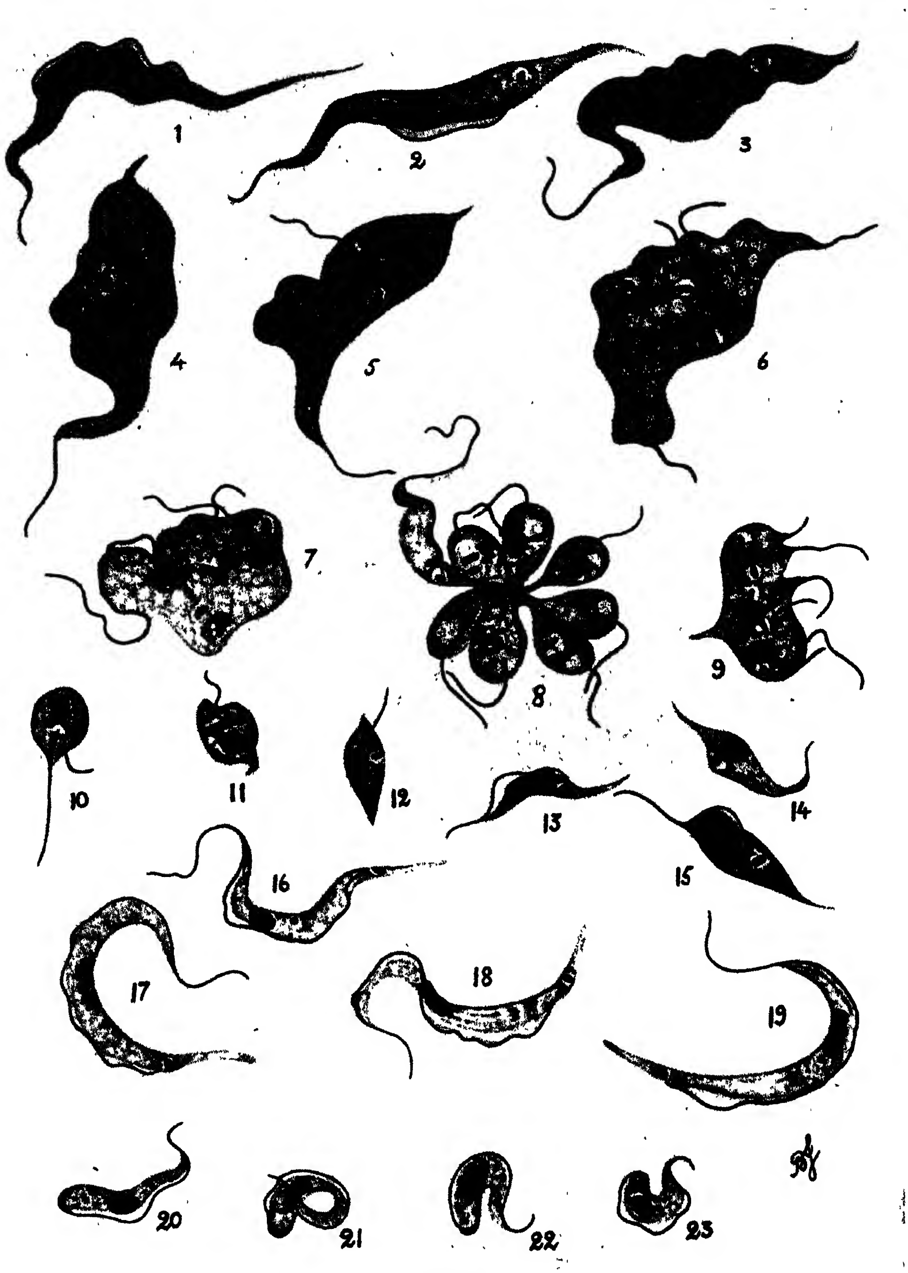


FIG. 197.—*Trypanosoma lewisi* (×2,000). (ORIGINAL.)

1-15. Forms which occur in the blood of the rat during the reproducing phase.
16-19. Forms which occur in the blood in the later stages of an infection.
20-23. Metacyclic trypanosomes which occur in the fæces of infective fleas.

Before it has become completely detached, division may again commence in the parent form, and the process may be repeated several times, so that a large individual, now, however, much reduced in breadth, with several small ones not completely separated, may occur (Fig. 197, 4–9). These small forms detach themselves, and may in turn divide more or less equally (Fig. 197, 10–11). On the other hand, the small forms may become round, and, while increasing in size, the kinetoplast divides repeatedly, together with the nucleus, the division of the latter being always a little behind that of the former, while new axonemes grow out from the newly-formed kinetoplasts. Cytoplasmic bodies are in this way produced which have 2, 4, 8, or 16 nuclei and kinetoplasts, and a corresponding number of axonemes and flagella (Fig. 197, 6–7). The nuclei are peripherally arranged, and the body becomes indented between the nuclei, and finally segmented into a number of organisms, which resemble the round parent form from which they were derived. Eventually, these small individuals elongate and become transformed into the trypanosome forms. The multiplication forms gradually disappear from the blood, and are replaced by the typical trypanosomes, which appear no longer to multiply. During the multiplication phase the various forms met with are referable to the types described, but all intermediate stages between these are met with, and a blood-film made at this period shows a wonderful series of organisms belonging to the various leishmania, leptomonas, crithidia, and trypanosome types described above. The origin of the large trypanosomes which commence the reproductive phase is doubtful. It is probable that they are the result of growth of the inoculated forms, which are those which occur in the late phase of an infection.

Reaction to Sera.—Laveran and Mesnil (1901*a*) first demonstrated that the serum of rats which had acquired immunity to *T. lewisi* after recovery from an infection had a marked agglutinating effect on the trypanosomes if blood containing them were mixed with the immune serum. In a few minutes the trypanosomes attached themselves to one another by their posterior ends, producing finally clumps of organisms (Fig. 152). The trypanosomes in these clumps are quite active, and the condition of agglutination may pass off, the individual trypanosomes swimming away. In other cases, if the agglutination persists, the trypanosomes eventually cease their movements and degenerate. There is evidence which indicates that two distinct substances are involved—an agglutinin and a trypanolysin. In some instances an auto-agglutination has been observed in the blood of infected animals. According to Taliaferro (1923, 1924), the serum of rats in the late stages of an infection contains a substance which inhibits the development of the trypanosomes. If 2 c.c. of such a serum is mixed with washed trypanosomes and injected

intravenously into healthy rats, no multiplication of the trypanosomes occurs, and no infection results, whereas, if the same experiment is conducted with the serum of a normal rat, the trypanosomes multiply and infection results in the usual manner. Coventry (1925) could not detect this substance in the blood of rats before the fifth day of an infection, though it is undoubtedly present, as reproduction is declining before this. There is a rapid increase in the quantity present in the blood between the fifth and sixth days, and a more gradual one up to the thirty-fifth day, after which it decreases up to the time when the infection ends.

Culture.—*T. lewisi* is readily cultivated in blood-agar media, and can be maintained for indefinite periods by subculture. In these cultures, the trypanosome forms disappear till every type of organism between leishmania and crithidia forms are met with, and large clusters of flagellates of all kinds are formed in which the organisms are arranged with their flagella directed inwards towards the centre of the mass. Delanoë (1911) showed that in old cultures trypanosome forms tend to reappear. They differ in structure from those originally introduced, and bear a striking resemblance to the infective metacyclic trypanosomes which are produced in the rectum of fleas. This observation lends support to the view that the type of development which occurs in the culture tube is an imitation of the invertebrate cycle of the trypanosome.

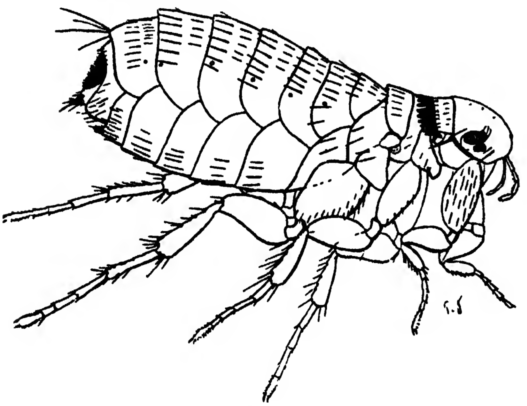


FIG. 198.—*Ceratophyllus fasciatus*, THE TRANSMITTER OF *Trypanosoma lewisi* (× 20). (ORIGINAL.)

The cultural form of *T. lewisi* will infect rats, though after long maintenance by subculture its power of doing so becomes diminished.

Pathology.—In its normal condition *T. lewisi* is not pathogenic to rats, which naturally recover from their infections. In accordance with this, practically no change is produced in the organs. In the strains of heightened virulence studied by Roudsky (1910–1911) degenerative changes with enlargement of the organ and lymphoid infiltrations occur in the liver and spleen.

Transmission.—That *T. lewisi* was transmissible from rat to rat by fleas was first proved by Rabinowitsch and Kempner (1899), who infected

rats by transferring to them fleas (*Ceratophyllus fasciatus*) taken from infected animals (Fig. 198). Swingle (1911) also conveyed infection by means of fleas (*C. lucifer* and *Pulex brasiliensis*), but the exact mechanism of infection was first definitely established by the work of Nöller (1912*d*), the writer (1913*b*), and Minchin and Thomson (1915), though Swellengrebel and Strickland (1910) had previously proved that infection was not conveyed by the flea in the act of biting, and had described the course of development in the flea which terminated in the production of the small metacyclic trypanosomes in the rectum. It is now known that infection takes place by uninfected rats eating the dejecta of fleas, or the fleas themselves, which have previously fed on infected rats (Fig. 199). Fleas do not become infective till after the lapse of about six days from the time of their feed on infected blood, during which interval a definite cycle of development takes place in the intestine. Yamasaki (1924) claims that the dog flea is able to infect by its bite as a result of regurgitation of trypanosomes which occur in the stomach and proventriculus, and that this method is as effective as the fæcal method of transmission.

Minchin and Thomson's method of experiment was to introduce clean rats into a cage of fleas which had previously had an opportunity of feeding on an infected rat. After remaining in the cage for about three days the rats were removed and exposed to chloroform vapour for a short time to immobilize the fleas upon them. The fleas were removed from the rats and returned to the cage. The course of the infection in the rats was then studied. The development in the fleas was traced by exposing clean fleas to infection from an infected rat, and examining them after various intervals.

Cycle in the Flea.—As already remarked, the main outlines of the developmental cycle in the flea culminating in the production of metacyclic trypanosomes in the rectum was first described by Swellengrebel and Strickland (1910), and Swingle (1911), while the mechanism of infection was established by Nöller (1912*d*) and the writer (1913*b*). Minchin and Thomson (1911) discovered the intracellular stage in the stomach of the flea, an observation confirmed by Nöller (1912*d*). Minchin and Thomson (1915) published a detailed account of the complete developmental cycle in the flea, and the experiments which led them to accept the view that infection of the rat was brought about by its ingesting the excreta of the flea. Further experiments on the mechanism of transmission with the dog flea, in which he claims that infection may be brought about by the bite, have been conducted by Yamasaki (1924).

The trypanosomes, which are of the type seen in the late phase of an infection in the rat, are taken into the stomach of the flea, where during the first six hours they undergo a change, which, however, is chiefly a

physiological one, in that they cease to bring about infection if injected into rats (Fig. 200, 1–4). They appear to become more rigid in character, and possibly more violent in their movements. At about the end of this period invasion of the lining cells of the stomach takes place (Fig. 20, 4–12). Actual penetration was observed by Nöller (1912*d*), who saw a

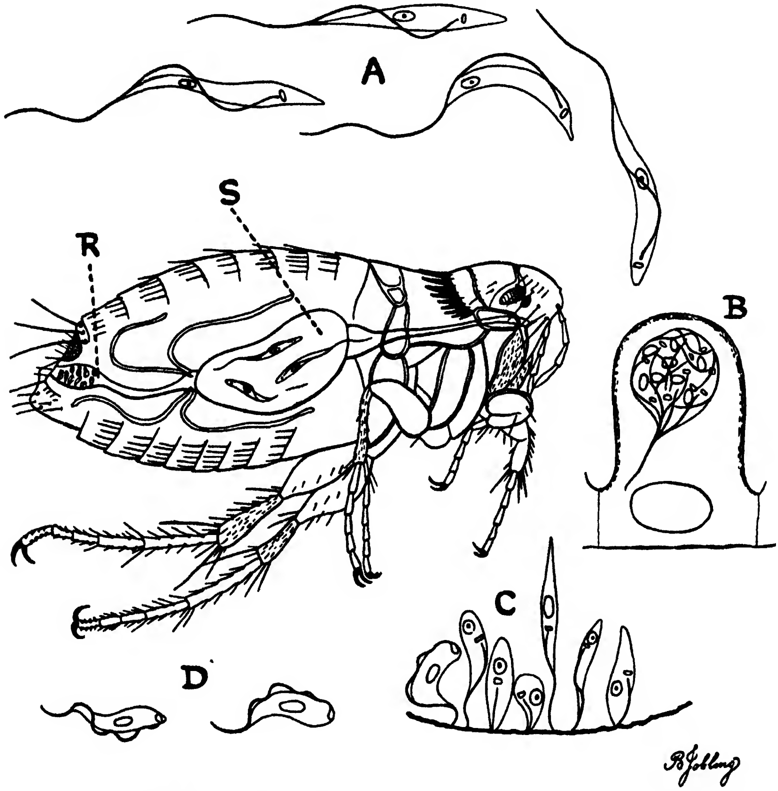


FIG. 199.—DIAGRAM OF *Trypanosoma lewisi* IN THE BLOOD OF THE RAT AND IN THE FLEA. (AFTER WENYON, 1922.)

A. Trypanosomes as seen in the rat at late phase of infection (× 1,500).
S. Trypanosomes in stomach of flea.
B. Intracellular phase of development in stomach (× 1,500). R. Rectal phase.
C. Attached flagellates in rectum: evolution of crithidia into metacyclic trypanosome form (× 1,500).
D. Free metacyclic trypanosomes which bring about infection when ingested by rat (× 1,500).

trypanosome enter the cell by its posterior end. Within the cell a vacuole forms, in which the trypanosome may be seen to exhibit active movements. It becomes doubled on itself, the two limbs of the U thus formed merging into one another to form a pear-shaped body. The volume of this pear-shaped body appears to be less than that of the trypanosome that entered

the cell, so that a reduction in size seems to have taken place. The pear-shaped body now grows in size, while the kinetoplast and nucleus multiply by repeated divisions. New flagella are formed from axonemes which develop from the daughter kinetoplasts, while the original flagellum still persists with its axoneme attached to one of the kinetoplasts. The bodies produced were described by Minchin and Thompson as " spheres." They may be spherical, with the flagella arranged irregularly about the surface of the " sphere," or the flagellar end of the original parasite may still survive, while the new flagella are arranged parallel to it, forming a tuft of bunched flagella. In the living condition the " spheres " are in constant motion. The number of nuclei and kinetoplasts produced is generally eight to ten, but there may be as many as fourteen. The diameter of the fully-developed "sphere" is 8 to 10 microns, and it finally divides into a number of trypanosomes which bear a striking resemblance to the original forms taken up from the rat's blood. The invaded cell is often reduced to a mere membrane enclosing the actively moving trypanosomes. It is suggested that the periplast of the original trypanosome contributes to the formation of this membrane. By rupture of the cell the trypanosomes escape into the stomach of the flea (Fig. 200, 11–12). Sometimes several " spheres " are developed in a single cell. The intracellular phase of development occurs in all parts of the stomach, and, commencing about six hours after the feed, it may cease as early as eighteen hours or persist as long as four or five days. The trypanosomes which escape by rupture of the cell may again enter other cells and repeat the process, but how many times this may occur is not known. It is probably very variable.

The next stage is the migration backwards of the trypanosomes to the hind-gut and rectum (Fig. 200, 12). These forms, which have pointed posterior ends and the kinetoplasts near but still posterior to the nuclei, are evidently approaching the crithidia form. Minchin and Thompson distinguish them as *crithidiomorphic* forms. Change in structure, which may have commenced before the trypanosomes actually leave the stomach, now takes place. This consists in a loss of activity, shortening of the body with rounding of the posterior end, diminution in length of the flagellum, and transposition of the nucleus and kinetoplast to give the true crithidia structure. Multiplication by fission of these crithidia forms takes place, and there then ensues the established rectal phase, in which a great variety of forms occurs (Fig. 200, 13–19). There are the typical short attached (haptomonad) forms, the free-swimming (nectomonad) crithidia forms, and finally the trypanosome forms. The small attached or haptomonad forms are derived from the trypanosomes which migrated from the stomach, and they give rise to the small infective trypanosomes (Figs. 197, 20–23, and 200, 19, *T*). The attached forms multiply, as do also the free-swimming

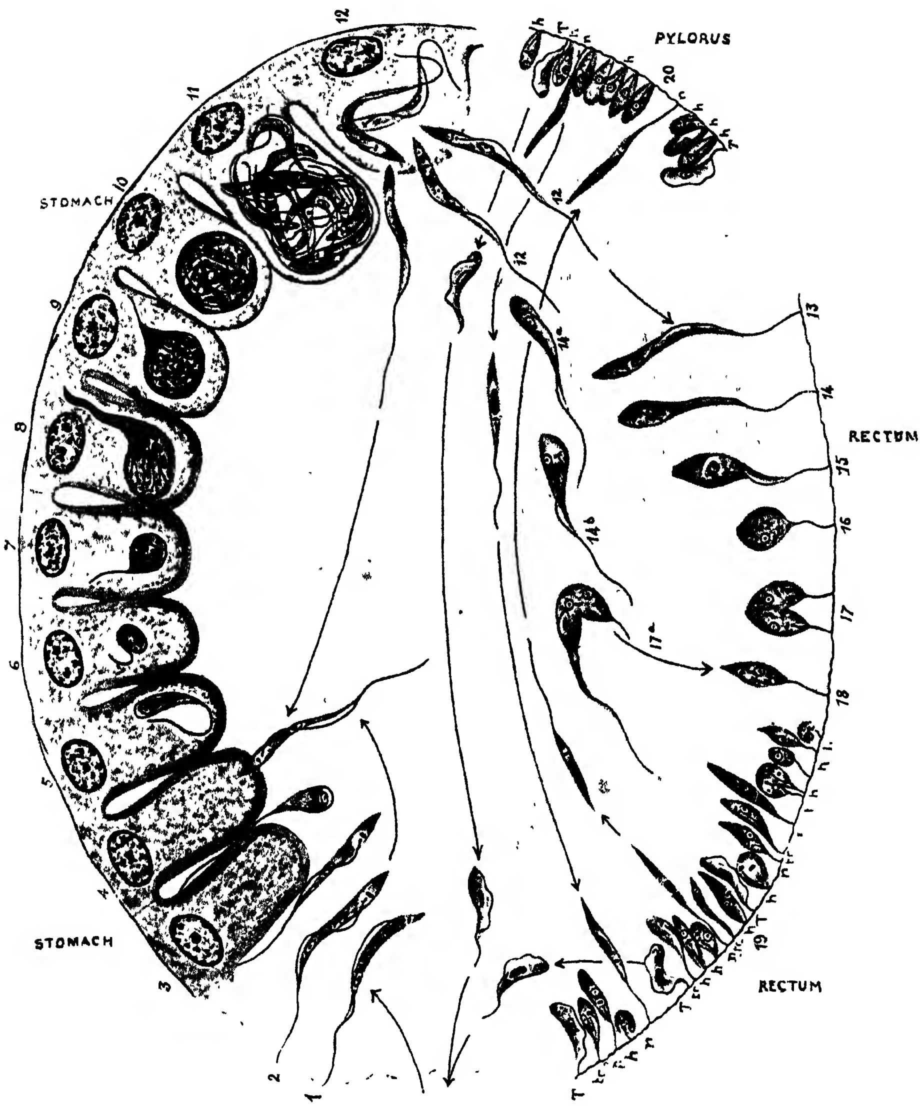


Fig. 200.—Diagram of Life-Cycle of *Trypanosoma lewisi* in the Flea (× 2,000). (After Minchin and Thomson, 1915.)

1. Trypanosome from rat's blood.
2. Slightly modified trypanosome after few hours in flea's stomach.

3-12. Stages in intracellular multiplication.

13-18. Two ways in which established rectal phase may arise from the stomach trypanosomes.

19. Established rectal phase, showing haptomonads (*h*), nectomonads (*n*), transitional crithidia types (*t.r.*), and metacyclic trypanosomes (*T*).
20. Secondary infection of the pyloric region of the hind-gut, showing forms similar to those which occur in the rectum.

crithidia forms, till the whole of the rectum may be covered with organisms. All these forms appear in the fæces of the fleas, but it is probable that it is only the small trypanosomes which bring about infection. Though the main development and attachment takes place in the rectum, this may also occur, but to a smaller extent, at the anterior end of the hind-gut near the pyloric opening.

Though Swellengrebel and Strickland (1910) had established the fact that rats could not be infected by the bites of the flea, the exact mechanism of infection was not understood till Nöller (1912*d*) published the results of his experiments. This observer employed the convenient method of handling individual fleas by tethering them on fine wire, a procedure adopted by showmen. By its use the movements of a flea can be completely controlled. Nöller's results were confirmed by the writer (1913*b*), using the wire method, and later by Minchin and Thompson (1915) with untethered fleas. Nöller found that the fleas repeatedly passed fæces or blood during the act of feeding, and that this could be collected and examined. About six days after the flea had fed on an infected rat, the small infective trypanosomes, as well as other forms, appeared in its fæces. Fleas in this condition were allowed to feed on uninfected rats, care being taken to prevent the voided fæces contaminating the skin. The fæces ejected were received on a cover-glass held behind the flea while feeding, and were transferred at once to the mouth of another rat. This experiment, repeated many times, always resulted in infection of the second rat and never the one bitten. Observing rats on which free fleas were placed, it was noted that the latter had the habit of congregating about the root of the tail, where they would feed when the rat was asleep. Aroused by their bites, the rat turns its head to allay the irritation or dislodge the fleas, which, startled by its movements, eject their fæces and escape into the fur. The freshly-passed fæces are then easily licked up by the rat. In this manner, by fleas passing from infected to uninfected rats, *T. lewisi* is transmitted in nature. Minchin and Thompson, working with *Ceratophyllus fasciatus* in the free condition on rats, noted that only a small percentage actually became infected. In the case of the fleas used by the writer, all became infected after feeding on an infected rat. Yamasaki (1924) obtained similar results.

This mode of transmission was demonstrated by Nöller (1912*d*) in the case of the dog flea, *Ctenocephalus canis*, and by the writer (1913*b*) for this flea, as well as the human flea, *Pulex irritans*, and the Indian plague flea, *Xenopsylla chæopis*. Minchin and Thompson (1915) proved it for the European rat flea, *Ceratophyllus fasciatus*, so that it is clear that many species of flea may act as vectors of *T. lewisi*. Furthermore, the complete development may take place in fleas which in nature rarely, if ever, have

an opportunity of feeding on rats. For instance, Brumpt (1913) showed that the swallow flea, *C. hirudinis*, might serve as a host for *T. lewisi*, and that the fæces of the fleas were infective to rats in the usual manner, while Nöller (1912*d*) showed that the development could take place in *Ctenopsylla musculi*.

It is highly probable that in nature infection may take place by rats actually devouring the infected fleas themselves.

From the above description it will be seen that the development in the flea consists of an intracellular multiplication phase in the stomach, followed by the transformation of the trypanosomes into crithidia forms and their migration to the rectum, where the attached phase results. Eventually, after the expiry of six days from the time of feeding, small metacyclic trypanosomes are voided in the fæces and ingested by the rats. It is important to note that at no stage was a sexual process encountered. Yamasaki (1924), who claims that the dog flea can transmit the trypanosome by its proboscis, also states that the intracellular stage is not essential to complete development in the flea.

Possible Transmission by Other Arthropods.—In addition to the experimental work with fleas, a good deal of attention has been paid to the rat louse, *Hæmatopinus spinulosus*. Prowazek (1905) described a developmental process, including syngamy, in the louse, leading to infection, not only of the gut, but also the body cavity fluid. The trypanosomes were supposed to be inoculated to the rat by the bite of the louse. Subsequent observation has not confirmed the developmental cycle, though it has been definitely shown by McNeal (1904), Nuttall (1909), and Baldrey (1909) that infection can be conveyed to rats by transferring lice from infected animals. Nöller (1914) studied the question of louse transmission, and was unable to find any evidence in favour of Prowazek's sexual phase, nor of the invasion of the body cavity fluid or biting organs. According to him, *T. lewisi* undergoes changes in the intestine of the louse, which are comparable to the culture of the trypanosome in artificial media. No established infection is produced in them as in the case of the flea, which, once infected, remains so for the rest of its life owing to continued multiplication of the attached forms in the rectum. The fæces of lice which have ingested infected blood will produce infection if eaten by the rat, as also will the louse itself, and Nöller thinks that in nature the louse may convey the trypanosome in a mechanical manner by being devoured while it still has trypanosomes within it, though as a vector it is of little importance compared with fleas. Several observers have shown that rats can be infected by feeding them with the blood or organs of infected rats.

It has been shown by the writer (1912*c*) and others that *T. lewisi* will

undergo changes comparable to those seen in artificial culture in blood media in the stomach of bed bugs and other arthropods, where a comparatively large quantity of blood is taken in and only slowly digested. This condition must not be mistaken for true infection. It has been a constant source of errors in experimental work with flagellates and biting arthropods. In many cases it may be difficult to decide between cultural developments and true infections, but in the latter the parasites tend to persist for long periods in spite of constant feeding, whereas, in the former, a second feed of blood often causes the flagellates to vanish. The experiments of Patton, La Frenais, and Rao (1921), referred to on p. 355, are of interest in this connection.

(*a*) Other Trypanosomes of Rodents.

The trypanosomes of rodents include the majority of forms known to occur in small mammals. The best known is *T. lewisi* of the rat, which has been dealt with above in some detail, and all the trypanosomes of this group resemble it closely. Species have, however, been created on slight differences in size, the failure of rats to become infected after inoculation, and the immunity of the hosts to infection with *T. lewisi.* In the few cases where it has been possible to study the complete development in the vertebrate, the resemblance to *T. lewisi* is very marked. The course of development in fleas in those cases which have been investigated is also identical with that of *T. lewisi.* It is possible that most, if not all, of these forms represent races of *T. lewisi* which have become adapted to particular hosts. It is evidently impossible to place reliance on differential characters which are based on slight morphological variations, especially when it is remembered that in the case of *T. lewisi* what were merely different stages of development of this trypanosome have been given special specific names.

Trypanosoma duttoni Thiroux, 1900.—This is a trypanosome which occurs in mice (*Mus morio* and *M. musculus*) in various parts of the world. According to Laveran and Mesnil (1912), in dimensions and method of multiplication in the mouse it closely resembles *T. lewisi* (Fig. 201, 9). Though easily inoculable from mouse to mouse, rats and guinea-pigs are not infected. Roudsky (1912), however, was able to increase its virulence till it was inoculable to rats, just as he raised the virulence of *T. lewisi,* as shown above, till mice became susceptible. Brumpt (1913) was able to demonstrate that *T. duttoni* had a cycle of development in the swallow flea, *Ceratophyllus hirudinis*, like that of *T. lewisi.* In the fæces of the fleas were found the small infective trypanosomes, and seven mice which were fed with the fæces became infected. The swallow flea can hardly be the natural host of the mouse trypanosome, yet in this flea its

development is apparently completed. *T. musculi* Kendall, 1906, is probably the same trypanosome.

T. avicularis Wenyon, 1909, from the zebra mouse (*Arvicanthus zebræ*), is of the *T. lewisi* type (Fig. 201, 11). It was discovered in the Sudan.

T. acomys Wenyon, 1909, of the spiny mouse (*Acomys* sp.), was described by the writer in the Sudan (Fig. 201, 12-13). It resembles *T. duttoni*, but is somewhat larger. The complete development was not studied.

T. grosi Laveran and Pettit, 1909.—This is a parasite of the field mouse, *Mus sylvaticus*. It was probably first seen by Gros in Russia in 1845. It is of the *T. lewisi* type, but is not inoculable to other animals. Laveran and Mesnil (1912) state that mice which had recovered from an infection were found to be sometimes inoculable with Roudsky's virulent strain of *T. lewisi*. The multiplication forms have not been seen.

T. microti Laveran and Pettit, 1909.—The host of this trypanosome is the field vole, *Microtus arvalis*. It is very active and of the *T. lewisi* type. The reproductive stages have not been described (Fig. 201, 10).

T. blanchardi Brumpt, 1905.—This trypanosome was discovered by Brumpt in the dormouse, *Myoxus nitela*. Its dimensions and development in the dormouse closely resemble those of *T. lewisi* in the rat. Brumpt (1913) was able to transmit it by means of the flea, *Ceratophyllus laverani*, the fæces of which contained infective trypanosomes. The trypanosomes seen by Galli-Valerio (1903) in the blood of *M. avellanarius*, and named by Blanchard *T. myoxi*, is possibly this species. *T. eliomys* França, 1909, is certainly identical with *T. blanchardi*.

T. evotomys Hadwen, 1912.—This trypanosome was discovered by Hadwen in the field mouse, *Evotomys saturatus*, in Canada. It resembles *T. lewisi*, but developmental stages were not described.

T. peromysci Watson, 1912.—This is another trypanosome of the *T. lewisi* type which occurs in the Canadian deer mice, *Peromyscus maniculatus*, *P. nebracensis*, and other species. The multiplication was not studied.

T. rabinowitschi Brumpt, 1906.—This form was discovered by Wittich (1881) in the hamster, *Cricetus frumentarius*. It closely resembles *T. lewisi*,

1. *T. vespertilionis* of the bat (*Pipistrellus pipistrellus*).
2. *T. megadermæ* of the Sudan bat (*Megaderma frons*).
3-4. *T. heybergi* of the Congo bat (*Nycteris hispida*).
5-6. *T. talpæ* of the mole (*Talpa europæa*).
7-8. *T. nabiasi* of the rabbit. 9. *T. duttoni* of the mouse.
10. *T. microti* of the field vole (*Microtus arvalis*).
11. *T. avicularis* of the zebra mouse (*Lemniscomys zebra*).
12, 13. *T. acomys* of the spiny mouse (*Acomys* sp.).
14-15. *T. legeri* of the sloth (*Tamandua tridactyla*).

FIG. 201.—VARIOUS TRYPANOSOMES OF SMALL MAMMALS (×2,000). (1, ORIGINAL; 2, 11, 12, 13, AFTER WENYON, 1909; 3 AND 4, AFTER RODHAIN, 1923; 5 AND 6, AFTER COLES, 1914; 7 AND 8, AFTER LAVERAN AND MESNIL, 1912; 19, AFTER THIROUX, 1905; 10, AFTER LAVERAN AND PETTIT, 1909; 14 AND 15, AFTER MESNIL AND BRIMONT, 1910.)

[*For description see opposite page*

but does not infect the rat. It is identical with *T. criceti* Lühe, 1906. Nöller (1912*c*) studied its development, and found it was morphologically identical with *T. lewisi*, but not inoculable to rats, mice, or guinea-pigs. Small trypanosomes, like the infective forms of *T. lewisi*, were found in the rectum of fleas, *Typhlopsylla assimilis*, *Ceratophyllus fasciatus*, and *Ctenocephalus canis*, which presumably are able to transmit the infection.

T. nabiasi Railliet, 1895.—This trypanosome, which Blanchard (1904) referred to as *Trypanosoma cuniculi*, occurs in rabbits, *Lepus domesticus* and *L. cuniculus* (Fig. 201, 7-8). It was first seen by Jolyet and Nabias (1891), and has been found by numerous observers in various parts of Europe. It is of the *T. lewisi* type and is not inoculable to rats and mice, but can be maintained in rabbits. The multiplication phase has not been properly studied. Brumpt (1913) proved its development in and transmission by the rabbit flea, *Spilopsyllus cuniculi*.

A trypanosome of the guinea-pig was described and figured by Kunstler (1898). Judging from the figure, it would seem that the organism was not a trypanosome at all.

Cazalbou (1913) claimed that he had discovered a large trypanosome in rabbits in France. It was 80 microns in length, the free flagellum being 10 to 12 microns long. There was a well-developed membrane. Though only one trypanosome was seen in one of a series of rabbits which died, the trypanosome was assumed to have been the cause of death. Cazalbou suggested the name *T. gigas* for this trypanosome. There seems to be considerable doubt as to the accuracy of this observation.

T. acouchii Brimont, 1909.—This is a trypanosome of the agouti (*Myoprocta acouchy*) of French Guiana, and is of the *T. lewisi* type. Two rats and two guinea-pigs were inoculated, with negative results. Multiplication forms are not known.

T. indicum Lühe, 1906.—This form occurs in the Indian palm squirrel (*Sciurus palmarum*). It resembles *T. lewisi*, but is distinctly smaller. Multiplication forms have not been seen.

T. spermophili Laveran, 1911.—This is a small trypanosome of the *T. lewisi* type, and is found in *Spermophilus musicus*, *S. guttatus*, and *S. eversmanni* of Russia and Siberia. The Canadian trypanosome *T. citelli* Watson, 1912, occurring in the squirrel, *Citellus richardsoni*, is possibly the same species.

T. otospermophili Wellman and Wherry, 1910.— This trypanosome is very similar to *T. spermophili*, and occurs in the Californian ground squirrel, *Otospermophilus beecheyi*. Neither this nor the last-named species has been fully studied.

T. bandicotti Lingard, 1904.—This trypanosome was discovered by Lingard in 1893 and named by him (1904). It occurs in the bandicoot

(*Nesokia gigantea*) of India, and closely resembles *T. lewisi*, from which it differs in that it is inoculable to guinea-pigs, in which it gives rise to fatal infections. The naturally infected animals are always young, a fact which suggests that an immunity is developed, as in the case of *T. lewisi* in the rat.

T. akodoni Carini and Maciel, 1915, in the South American rat, *Akodon fuliginosus*; *T. eburneensæ* Delanoë, 1915, of the West African rat, *Rattus couchar*; *T. guist'hani* Delanoë, 1915, of the Savannah rat, and *T. crociduræ* Brumpt, 1923, of the shrew, *Crocidura russulus*, of France, are all of the *T. lewisi* type, but in no case is the complete development known.

(*b*) Trypanosomes of Cheiroptera.

A trypanosome of the bat was first noted by Dionisi (1899*a*) in Italy in *Miniopterus schreibersii*. Donovan (quoted by Laveran and Mesnil, 1904) found trypanosomes in the large Indian bat, *Pteropus medius*. Battaglia (1904) gave the name *T. vespertilionis* to a trypanosome of *Vesperugo noctula*, while Ed. and Et. Sergent (1905) described *T. nicolleorum* and *T. vespertilionis* from the North African bats, *Myotis murinus* and *Vespertilio kuhli*. In the same year Petrie (1905) saw a trypanosome in the English bat, *Vesperugo pipistrellus*. It was found later in the same bat in other parts of Europe, while Bettencourt and França (1905) in Portugal found it in three species of *Vesperugo* (*V. pipistrellus*, *V. serotinus*, and *V. nattereri*), and named it *T. dionisii*. Cartaya (1910) described, under the name of *T. phyllostomæ*, a trypanosome of the American bat, *Phyllostoma perspicillatum*.

Laveran and Mesnil (1912) state that in their opinion all these various forms belong to *T. vespertilionis* Battaglia, 1904, which has a striking resemblance to *T. cruzi* (Fig. 201, 1). Nicolle and Comte (1908*b*), in Tunis, found *Vespertilio kuhli* to be commonly infected with the large and small trypanosomes described as separate species by Ed. and Et. Sergent (1905). They expressed the opinion that they both belonged to the one species, *T. vespertilionis*. Cultures on blood-agar medium were obtained, and these were easily carried on by subculture. Nicolle and Comte (1909) attempted to infect three young bats by means of the cultural forms, but no infections resulted. Laveran and Mesnil (1912) state that these observers succeeded in infecting one out of twenty bats inoculated. The writer (1909) described a larger trypanosome from the Sudan bat, *Megaderma frons*, under the name of *T. megadermæ* (Fig. 201, 2). It has a length of 40 microns, and is distinctly larger than the largest known forms of *T. vespertilionis*, which varies in length from 14 to 24 microns and in breadth 1 to 2 microns. Iturbe and Gonzalez (1916) described as *T. lineatus* a trypanosome seen by them in the Venezuelan bat, *Vampirops*

lineatus. It measured 19·5 microns in length, had a well-developed membrane and a central nucleus. According to them, it resembled *T. brucei* rather than *T. lewisi*. Leger and Baury (1923) describe as *T. morinorum* a trypanosome of the bat (*Hipposiderus tridens*) of Senegal. It is broader than *T. vespertilionis*, and measures 30 by 7 to 8 microns. The part of the body behind the kinetoplast represents about half the length of the body. The kinetoplast is close to the nucleus, which is centrally placed. There is a free flagellum of 7 to 15 microns in length. A closely allied form is *T. heybergi*, which was discovered by Rodhain (1923) in the insectivorous bat, *Nycteris hispida*, of the Belgian Congo (Fig. 201, 3-4). It is also a broad trypanosome, but differs from *T. morinorum* in some of its dimensions.

FIG. 202.—*Trypanosoma vespertilionis* (=*Schizotrypanum pipistrelli* CHATTON AND COURRIER, 1921) OF THE BAT, *Vesperugo pipistrellus*. (AFTER CHATTON AND COURRIER, 1921.)

1. Two cysts in the mucosa of the intestine (× *ca.* 100).
2. Two cysts in the stroma of the ovary, one of which (*a*) contains crithidia forms, and the other (*b*) trypanosomes (× *ca.* 500).
3. *a* and *b*, Trypanosomes from the blood; *c*, crithidia form from the tissues; *d*, cultural form of trypanosome (× *ca.* 2,000).

As regards the various trypanosomes mentioned above, it is at present impossible to decide whether those that have been given specific names are good species or not. In no case has the complete development been studied, and nothing is known of the range of variation of the blood forms of any one of them. The work of Chatton and Courrier (1921) shows that the life-history may be a very complicated one. These observers have described, under the name *Schizotrypanum pipistrelli*,

a trypanosome of *Vesperugo pipistrellus* of Alsace. As they admit, they have little evidence to indicate that they were not dealing with *T. vespertilionis*, except that in this instance they discovered a somewhat remarkable developmental process which had not been previously observed (Fig. 202). By cutting sections of various organs of infected bats they noted that the trypanosome reproduces within cysts which may reach a diameter of 200 microns. In this respect it resembles *T. cruzi*, and is placed by them in the genus *Schizotrypanum*. As will be shown below, there is no actual reproduction by schizogony of *T. cruzi*, which multiplies by binary fission like all other trypanosomes, so that there is no valid ground for placing it in a separate genus. For the same reason the form described by Chatton and Courrier will be included in the genus *Trypanosoma*. The cysts referred to above were found in various situations—mucosa and submucosa of the stomach and intestine, the gall bladder, kidney, bladder, spleen, ovary, uterus, epididymis, and peritoneum. Within the cysts there occurred flagellates of various forms, but in any individual cyst all the flagellates were of the same type. The simplest forms seen were short stumpy crithidia forms. It appears as if multiplication occurs within the cysts by repeated division of these forms. When the cyst is mature the short forms increase in length, and finally become the typical trypanosomes, which escape into the blood by rupture of the cyst. It will be noted that in this trypanosome the reproducing forms are of the short crithidia type, whereas in the cysts of *T. cruzi*, to be described below, the multiplying forms are of the leishmania type. The trypanosomes which appear in the blood of the bat do not differ from *T. vespertilionis*, as described by other observers, so that it seems highly probable that Chatton and Courrier have observed the reproductive process in *T. vespertilionis* for the first time. Coles (1914) gave a description of *T. vespertilionis* of the English bat. He noted that in the heart blood there occurred, beside the typical trypanosomes, immature forms which from his microphotographs appear to have a close resemblance to the stumpy crithidia forms seen by Chatton and Courrier within the cysts. Very similar forms have been seen in smears of the liver and lung by Franchini (1921).

As regards the method of transmission of the trypanosomes of bats very little is known. Gonder (1910) discovered trypanosomes in the stomach of mites (*Liponyssus arcuatus*) taken off bats. He believed that the mite would be found to be thevector of *T. vespertilionis*. Nicolle and Comte (1909), however, suspected the bug, *Cimex pipistrelli*, which is frequently found on young bats in Tunis, and Pringault (1914) claims to have transmitted the trypanosome to four out of five bats by the bite of this bug. Bats were also infected by inoculating them with crushed bugs. Sergent, Et. and Ed. (1921*a*), have noted the occurrence of flagellates of the

leptomonas and leishmania types in this bug, and raise the question of their being developmental forms of the bat trypanosome. What are probably developmental stages of the trypanosome were seen by Franchini (1921) in the mite, *Leiognathus laverani*. Rodhain (1923) found that mites (*Leiognathus*) taken from infected bats harboured crithidia and trypanosomes, so that it seems probable that this mite is the vector of the trypanosome named *T. heybergi* by Rodhain.

Battaglia (1914) has claimed that *T. vespertilionis* is pathogenic to rabbits. He makes a similar claim for *T. lewisi*. No other observer has succeeded in confirming these statements, attempts at infecting laboratory animals with the trypanosomes of bats having invariably failed.

(*c*) Trypanosomes of Insectivora.

Trypanosoma talpæ Nabarro, 1907.—Petrie (1905) discovered a trypanosome in the English mole, *Talpa europæa*. The trypanosome was again seen by Thomson, J. D. (1906), and by França (1911*a*) in Portugal in *T. europæa* and *T. cæca*. Though resembling *Trypanosoma lewisi* in some respects, it is not inoculable to rats (Fig. 201, 5-6). Nabarro (1907) gave it the name *T. talpæ*. Laveran and Franchini (1913*b*) found developmental forms of the trypanosome in the mole flea (*Palæopsylla gracilis*).

T. soricis Hadwen, 1912.—This is a trypanosome of the wandering shrew (*Sorex vagrans*) in Canada. It is of the *T. lewisi* type, but reaches a total length of only 17·5 microns.

T. brcdeni Rodhain, Pons, Vandenbranden and Bequært, 1913.—This form, again, is of the *T. lewisi* type, and occurs in *Petrodromus tetradactylus* of the Belgian Congo.

T. denysi Rodhain, Pons, Vandenbranden and Bequært, 1913.—This trypanosome, which is larger than the preceding one, was discovered in *Pteromys volans*. It had a total length of 37 to 48 microns, of which 8 to 10 microns represented the flagellum.

T. xeri Leger and Baury, 1922.—This form occurs in the fossorial squirrel (*Xerus erythropus*) of Senegal, and is very similar to *T. denysi*.

(*d*) Trypanosomes of Edentata.

A trypanosome, named *T. legeri* by Mesnil and Brimont (1910), was discovered by Brimont in an ant-eater, *Tamandua tridactyla*, in French Guiana (Fig. 201, 14-15). The body of the trypanosome is 30 to 35 microns in length, and the flagellum 10 to 13 microns. In breadth it varies on either side of 5 microns. The posterior extremity extends for about 14 to 16 microns beyond the kinetoplast. Besides these large forms there occurred others which were smaller, and resembled *Trypanosoma*

lewisi in shape and dimensions. The undulations of the membrane are more marked than in the rat trypanosome. Mesnil and Brimont (1908*a*) described a trypanosome in another edentate (*Cholœpus didactylus*) in the same locality which may be identical with *T. legeri*. It occurred in the blood in association with *Endotrypanum schaudinni* (p. 485).

(*e*) Trypanosomes of Carnivora.

Trypanosoma pestanai Bettencourt and França, 1906.—This trypanosome occurs in the badger, *Meles taxus*, of Portugal. It has a breadth of 5 to 6 microns and a total length of 30 to 32 microns. The posterior extremity is prolonged beyond the kinetoplast for a considerable distance, and there is a flagellum 4·3 microns in length. The membrane is well developed.

A trypanosome was seen by Fehlandt (1911) in an otter in Tanganyika, and one in a lion by Weck (1914) in East Africa. In both these cases it is supposed the trypanosomes were of the pathogenic forms of Africa.

(*f*) Trypanosomes of Monkeys.

Trypanosoma prowazeki Berenberg-Gossler, 1908.—This trypanosome was discovered by Berenberg-Gossler in a monkey (*Brachyurus calvus*) from the Amazon district (Fig. 203, 1). It measured (flagellum included) 21 microns in length by 2 microns in breadth. The flagellum was 7 microns long. Laveran and Mesnil (1912) regard it as allied to *T. cruzi*.

T. minasense Chagas, 1909.—This trypanosoma, first seen by Chagas, appears to be a common parasite of marmosets, *Hapale penicillata* and *H. jacchus*, of South America (Fig. 203, 2). The body of the trypanosome measures 30 to 35 microns in length, and there is a free flagellum 8 to 10 microns long. The breadth is 4 to 6 microns.

T. vickersæ Brumpt, 1909.—This form was discovered by Brumpt (1919*b*) in *Macacus cynomolgus* (Fig. 203, 4-5). Its length is 20 to 22 microns, of which the flagellum occupies about 8 microns. In general structure and pathogenicity it resembles *T. cruzi*. It was inoculable to *M. cynomolgus* and to other monkeys, *M. rhesus* and *M. sinicus*, as also to rats, mice, guinea-pigs, dogs, and marmosets. The same trypanosome appears to have been discovered in a *M. rhesus* at the Rockefeller Institute by Terry (1911), who proposed to name it *T. rhesi*.

A very similar, if not identical, trypanosome which bears some resemblance to the established forms of *T. lewisi* was found in *M. sinicus* in Algiers by Et. Sergent (1921). The trypanosome was not seen on direct blood examination, but was obtained in culture in N.N.N. medium, in which it grew very readily.

T. lesourdi Leger and Porry, 1918.—This trypanosome occurs in the

monkey, *Ateles pentadactylus*, of French Guiana. It is a small trypanosome with a body 14 microns in length and a flagellum 5 microns long. The kinetoplast is large and round, and situated 3 microns from the posterior extremity. There is a well-developed membrane.

T. devei Leger and Porry, 1918.—This form was found in *Midas midas* in French Guiana. It is a long, thin trypanosome, the body of which measures 37 microns and the flagellum 7 microns. The breadth is 2 to 2·5 microns. The kinetoplast is some distance from the posterior end of the body, and there is a well-developed membrane. It is of the *T. lewisi* type.

Brimont (1909) discovered a trypanosome in a howler monkey (*Alouatta senicula*) captured in French Guiana (Fig. 203, 3). Only a single trypano-

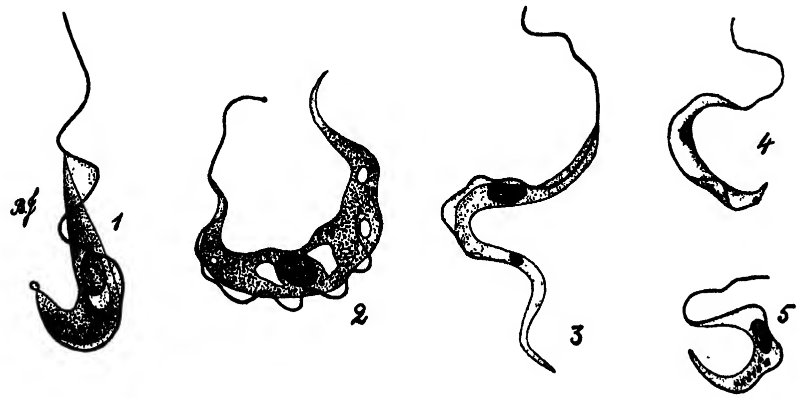


FIG. 203.—TRYPANOSOMES OF MONKEYS (×2,000). (1, AFTER BERENBERG, GOSSLER, 1908; 2, AFTER CARINI, 1909; 3, AFTER BRIMONT, 1912; 4 AND 5, AFTER LAVERAN AND MESNIL, 1912.)

1. *T. prowazeki* of the Ouakasi monkey (*Ouakasi calvus*).
2. *T. minasense* of the marmoset (*Hapale penicillata*).
3. *T.* sp. of the howler monkey (*Alonatta senicula*).
4-5. *T. vickersæ* (*Macaca fascicularis=M. cynomolgus*).

some was seen, and it had a length of 28 microns, of which the flagellum occupied 9 to 10 microns.

In Africa, in endemic centres of sleeping sickness, trypanosomes have been noted by several observers in monkeys. They have generally been regarded as *T. gambiense*. Ziemann (1902*a*) recorded a trypanosome in a chimpanzee in the French Congo, Kudicke (1906) a large trypanosome in *Cercopithecus* sp. in German East Africa, and Dutton, Todd, and Tobey (1906) one from *C. schmidti* of the Belgian Congo, which measured about 25 by 2·5 microns. Martin, Lebœuf, and Roubaud (1909) saw a trypanosome in a lemur (*Galago demidoffi*) of the French Congo, while Koch, Beck, and Kleine (1909) observed a trypanosome in a captured monkey, and regarded it as *T. gambiense*, as also did Bruce *et al.* (1911*d*).

Reichenow (1917, 1920*c*) observed trypanosomes of the *T. lewisi* type in both chimpanzees and gorillas, as also in a lemur (*Perodictus*) and in *C. cephus* in the Cameroons. He proposed to name the trypanosome *T. lewisi* var. *primatum*, as on morphological grounds he regards it as a variety of *T. lewisi.* Yamasaki (1924), as already noted, failed to infect monkeys by means of fleas which had become infective after feeding on rats harbouring *T. lewisi.* Direct inoculation of blood from infected rats into monkeys has also failed to infect them with *T. lewisi.* Chagas (1924), in Brazil, found monkeys (*Chrysothrix sciureus*) naturally infected with trypanosomes. These were studied in inoculated guinea-pigs and dogs, with the result that he arrived at the conclusion that the trypanosome was *T. cruzi*, with which it agreed in its morphology and method of multiplication.

Genus: Endotrypanum Mesnil and Brimont, 1908.

Mesnil and Brimont (1908*a*) described under the name *Endotrypanum schaudinni* a curious parasite which occurred in the red cells of *Cholœpus*

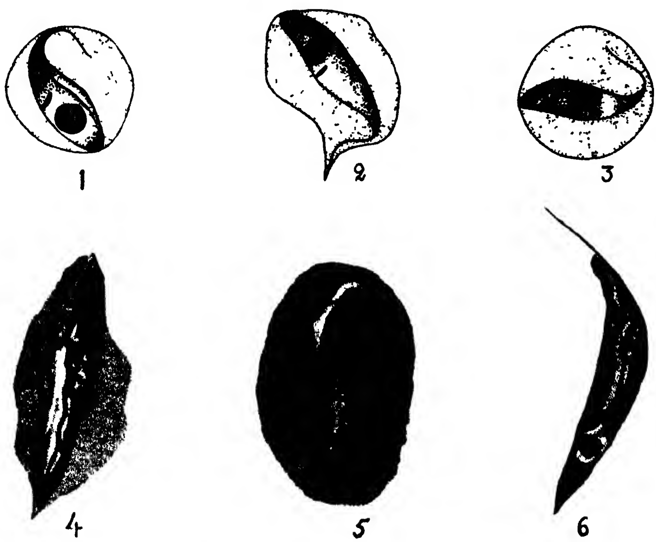


FIG. 204.—*Endotrypanum schaudinni* IN THE BLOOD OF THE SLOTH, *Cholœpus didactylus*. (1-3, AFTER MESNIL AND BRIMONT, 1908; 4-6, AFTER DARLING, 1914).

1-3. Parasites in the red blood-corpuscles (× *ca.* 1,800).
4-6. Two intracorpuscular forms and one free form (× *ca.* 3,000).

didactylus, the two-toed sloth of Guiana (Fig. 204). As it is undoubtedly related to trypanosomes, it is considered here. It was elongated and piriform in shape, one end being blunt or rounded and the other fine and

tapering. It was longer than the diameter of the corpuscle, and either pushed this out at one point or was curved to adapt itself to the space available. In the stained films it consisted of blue staining cytoplasm, and possessed a large, round, red nucleus, by the side of which was a rod-like body. As a trypanosome occurred in the blood at the same time, the possibility of these bodies being intracorpuscular stages of the trypanosome naturally occurred to the observers. The parasite did not possess a flagellum, and no axoneme was visible. It measured 8 to 11 microns in length by 2·5 to 4 microns in breadth. No free forms were discovered, and there did not occur any which could be considered as intermediate between the trypanosomes and the intracorpuscular parasites.

This curious organism was again seen by Darling (1914) in Panama. He had an opportunity of studying it in the living condition. The parasite was within the red cells immediately after the blood was taken. It showed active movements, and eventually liberated itself from the cell. One end was rounded and the other tapering, and in some there was a definite undulating membrane extending towards the pointed extremity. In stained specimens the nucleus and kinetoplast described by Mesnil and Brimont were seen, and in addition a filament running along one side of the organism. This was undoubtedly the axoneme. The general appearance of the parasite was that of a crithidia or cultural form of a trypanosome, to which it seems to be nearly related. Labernadie and Hubac (1923) also discovered the organism in Guiana. They noted both intracellular as well as free forms. In some there was a free flagellum 4 to 6 microns in length, while occasionally the kinetoplast was at the posterior end of the organism, giving the parasites a definite trypanosome structure. The organism was seen by the writer and Scott (1925*a*) in Brazilian sloths (*C. didactylus*) which had died in London.

II. THE TRYPANOSOME OF MAN IN SOUTH AMERICA.

Trypanosoma cruzi Chagas, 1909.—Synonyms: *Schizotrypanum cruzi* (Chagas, 1909); *T. escomeli* Yorke, 1920. This trypanosome, which produces a disease in man in South America, will be considered here, as it appears to be more nearly related to *T. lewisi* than to the other pathogenic trypanosomes of man and animals (Plate V., L, p. 456).

T. cruzi was first discovered by Chagas in 1907, and described by him (1909) as a parasite of the reduviid bug, *Triatoma megista*. The bugs were known to attack man in certain parts of Brazil, and Chagas discovered crithidia forms of a flagellate in the hind-gut of specimens of the bug collected at Minas. Some of these were allowed to feed on a marmoset, *Hapale penicillata*, which three weeks later showed trypanosomes in its

blood. The trypanosomes were found to be inoculable to dogs, guinea-pigs, and rabbits. Extending his observations, Chagas ultimately found the organism in a cat, and later in children who suffered from a wasting disease which had long been known in the country. Chagas first placed the trypanosome in the genus *Trypanosoma*, but later, on account of its

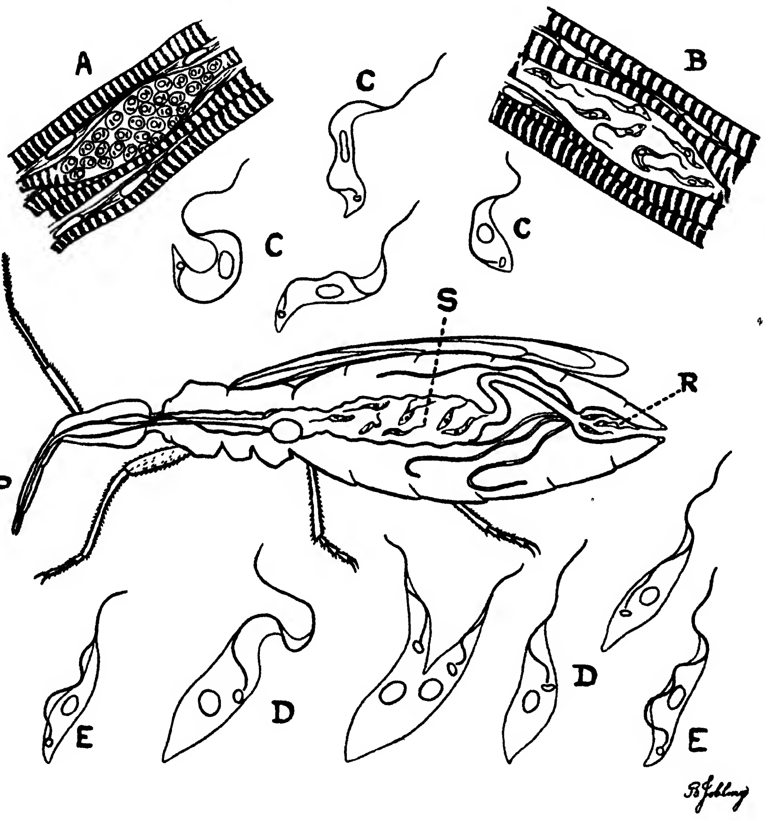


FIG. 205.—DIAGRAM OF *Trypanosoma cruzi* IN THE BLOOD AND TISSUES OF MAN AND IN THE BUG (*Triatoma megista*). (AFTER WENYON, 1922.)

A. Leishmania forms in muscle fibre of heart. B. Trypanosome forms in muscle fibre.
C. Trypanosome forms in blood. S. Trypanosomes in stomach of bug.
R. Rectal phase of development. D. Multiplying crithidia forms in rectum of bug.
E. Metacyclic trypanosome forms which produce infection. These forms usually have no flagella.

peculiar intracellular mode of development as leishmania forms, created the new genus, *Schizotrypanum*, for its reception. This name was chosen because it was believed that reproduction took place by schizogony, but it is now known that multiplication, though occurring within cells in the leishmania stage, is by the usual method of binary fission, so that it is preferable to retain the trypanosome in the genus *Trypanosoma*.

As already remarked, *T. cruzi* was first discovered in children at Minas in Brazil. Later it was shown to occur in other parts of Brazil also, and by Tejera (1919*a*) in the States of Zulia and Trujillo in Venezuela, and by Escomel (1919*a*) in Peru. As will be shown below, the infection in the reduviid bugs is much more widespread in South America than is the disease in human beings which is often termed Chagas' disease.

Symptomatology.—The disease has been described in detail by Chagas and other observers. It occurs in children of all ages, but assumes an acute form in the first year of life. In these cases the incubation period varies between ten days to a month. There is fever, wasting anæmia, enlargement of the liver, spleen, and lymphatic glands, and especially of the thyroid, producing a puffy condition of the face and body. A more chronic condition exists in older children, in which the above symptoms develop more slowly, while the involvement of the thyroid gland produces a pseudo-myxœdematous or a well-defined myxœdematous condition. The chronic form occurs in children up to fifteen years of age, and is associated with retarded development of mind and body. In any of these cases there may occur special symptoms attributable to involvement of the heart, meninges, or brain. The disease, though most commonly occurring in children, also attacks adults. *T. cruzi* does not occur in great numbers in the blood of infected individuals. As a rule there is a scanty infection, the parasite being found with difficulty on direct examination. It is more readily demonstrated by inoculation of blood into a susceptible animal like the marmoset or guinea-pig. It has also been found in the cerebro-spinal fluid. The reproducing forms occur in cells of various organs which are histologically altered by the parasites.

Pathology.—The pathological changes caused by the trypanosomes consist in the degeneration of the invaded cells, and a leucocyte invasion of the affected tissue in which numerous leishmania and other forms of the parasite occur (Fig. 206). There is an increase of fibrous tissue, often leading to definite sclerosis. This is especially well seen in the thyroid and ovaries. The changes are most marked in those organs most heavily invaded by the parasite, and neither in man nor animals is it possible to predict which part of the body will be most affected.

Morphology.—The trypanosome itself is a curved, stumpy organism with a sharp posterior end (Fig. 209, 1-3, and Plate V., K, p. 456). Its length, including the flagellum, varies on either side of 20 microns, but not to any great extent. Some individuals are broad and others narrow, and, as has been suggested in the case of other trypanosomes, this variation was supposed by Chagas to represent a distinction between female and male trypanosomes. The proof of this, however, is lacking. Brumpt

FIG. 206.—*Trypanosoma cruzi*: LEISHMANIA FORMS IN SECTIONS OF TISSUES OF HUMAN CASE (× *ca.* 1,000). (AFTER CHAGAS, 1916.)

1. Heart muscle. 2. Brain. 3. Thyroid.

(1912) believes that the narrow forms are the young ones escaped from the cysts, and that they gradually grow into the broader individuals. The nucleus is central in position, while the kinetoplast is a relatively large ovoid or egg-shaped body close to the pointed posterior end. The undulating membrane is narrow and only slightly convoluted. The flagellum represents about a third of the total length of the organism. The curved character of the short broad body with the large "egg-shaped" kinetoplast and comparatively straight membrane gives *T. cruzi* at this stage of its development a very characteristic appearance. Chagas described certain forms within the red blood-corpuscles, but this observation has not been confirmed, and it is probable he was merely dealing with superimposed trypanosomes or other structures. *T. cruzi* appears to be a peculiarly fragile organism, for in the process of making blood-films from infected animals many of the trypanosomes are damaged.

Escomel (1919*a*) described what he believed to be the first case of *T. cruzi* infection to be noted in Peru. In his description of the trypanosome he gave the length as 20 to 40 microns, and stated that the kinetoplast was not well developed. From the description, it appeared to Yorke (1920*a*) that Escomel must have been dealing with some trypanosome other than *T. cruzi*. He accordingly proposed to name it *T. escomeli*. In the following year Escomel (1920) gave a more detailed account of the trypanosome. He corrected his previous measurements, while from the figures he gave there is little doubt that he was actually dealing with *T. cruzi*, so that the name *T. escomeli* becomes a synonym.

Multiplication.—Longitudinally dividing forms of *T. cruzi*, such as are found in the blood in the case of other trypanosome infections, do not occur, and this is explained by the type of reproduction which was specially studied by Vianna (1911), and which bears a striking resemblance to the method of multiplication of the trypanosome of the bat, *Vesperugo pipistrellus*, as described by Chatton and Courrier (see p. 480). The multiplication of *T. cruzi* takes place within the cells of nearly every organ of the body—not only the endothelial cells of the capillaries and lymphatics, but also the organ cells themselves. In some cases one organ is more involved than another, a feature which accounts for the special symptoms seen in certain cases. The heart and voluntary muscles, the nervous system, thyroid, lymphatic glands, bone marrow, suprarenal capsules, ovaries, and testis have all been found invaded by the multiplying forms. The process can be readily studied in sections of the heart muscle and other organs of mice, rats, and guinea-pigs, or in smears made from these organs (Fig. 207). Multiplication appears to commence after the invasion of a cell by a single trypanosome which, losing its membrane and flagellum, becomes a leishmania form measuring

about 4 microns in diameter. This commences to divide by simple fission after division of its nucleus and kinetoplast (Fig. 207, 17-20). By repeated fissions in this manner intracellular cysts are produced which contain large numbers of leishmania forms. The cyst is more of the nature of a vacuole, as a definite wall is not present, the cell being enlarged and reduced to a

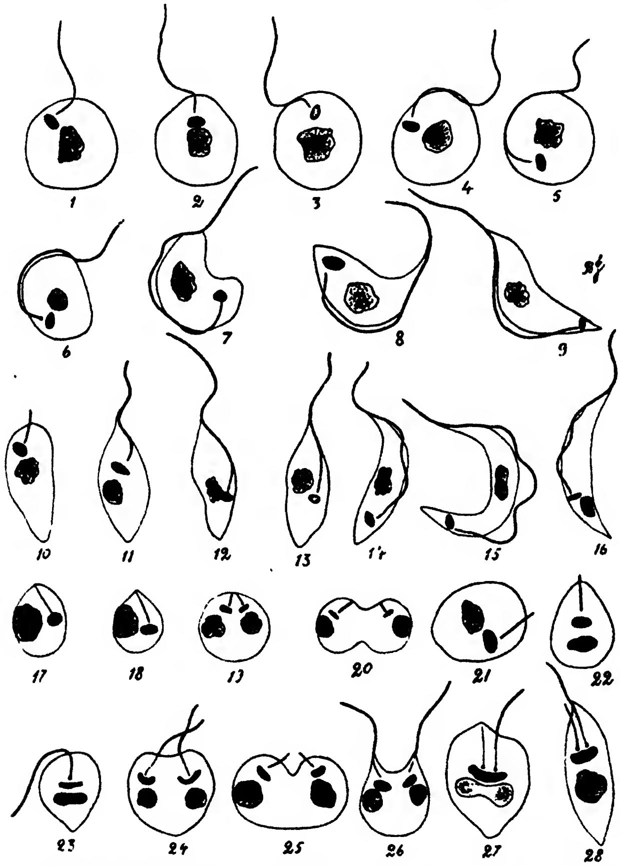


FIG. 207.—*Trypanosoma cruzi* IN SMEAR OF HEART OF A MOUSE (× 2,000). (ORIGINAL FROM PREPARATION MADE BY DR. TEJERA.)

1-9. Stages in development of a trypanosome from rounded flagellated stage.
10-15. Stages in development of a trypanosome from elongate flagellated stage.
16. Posterior nuclear trypanosome form. 17-20. Division of leishmania form.
21-23. Growth of flagellum in leishmania form. 24-28. Division of flagellated forms.

mere enclosing membrane with its nucleus flattened and pushed to one side. At a certain stage each of the leishmania forms develops a flagellum, and by gradual changes in the arrangements of its parts becomes transformed into a crithidia form, and finally into a trypanosome of the blood type (Fig. 207, 1-20). In any single group of organisms the change affects

all the individuals at the same time and at approximately the same rate, so they all arrive at maturity together. During the development of flagella and the transformation into trypanosomes division may still take place (Fig. 207, 24-28). Rupture of the cell liberates the trypanosomes, which escape into the blood-stream. According to Brumpt (1912), when they first enter the blood-stream they are very narrow, active trypanosomes which grow into the broader forms. A point which does not appear to be definitely decided is whether the infection of fresh cells is brought about by the blood trypanosomes entering new cells, and there becoming again transformed into leishmania forms, which recommence the division process, or whether new cells are infected by leishmania forms escaping from ruptured cells. In sections of the organs of infected mice or guinea-pigs, the writer has often seen ruptured cysts containing the leishmania forms, and isolated leishmania forms scattered amongst the cells, so that it does not seem improbable that they might continue the process of multiplication if taken into the cytoplasm of other cells. It might be supposed that the blood type is only capable of development in the invertebrate host, but this does not seem to be the case, for all the phases of reproduction of leishmania forms within the cells will commence after inoculation of an animal with blood containing the mature trypanosomes.

Chagas (1909) described a peculiar form of pulmonary reproduction in which small cysts are produced by the looping of a trypanosome into a U-shape and its concentration into an ovoid body. The kinetoplast is supposed to be thrown out, and the nucleus divided into eight small nuclei. Finally, the contents of the cyst divide into eight small merozoites, which are presumed to enter the red blood-corpuscles and develop into mature trypanosomes of the male and female type. This method of reproduction has not been confirmed, and there is little doubt that Chagas was dealing with another organism, probably *Pneumocystis carinii* (Fig. 450).

Hartmann (1910, 1917) has described a process of schizogony which commences by a single trypanosome becoming a leishmania form within a cell. Nuclear and kinetoplast divisions take place repeatedly, and by growth a large cytoplasmic body is produced containing many nuclei and kinetoplasts. Segmentation into separate leishmania forms then occurs. Hartmann also describes a schizogony stage in which the nucleus alone is present, the kinetoplast being absent. The figures given by Hartmann are far from convincing, and suggest the presence in a cell of numerous leishmania forms which have lost their outlines through degeneration. The schizonts appear to be portions of the cytoplasm of cells containing the nuclear remains of degenerating or badly fixed parasites. Similar appearances have led to the view that *Leishmania donovani* also reproduces by schizogony (p. 408).

Culture.—*Trypanosoma cruzi* cultivated in N.N.N. medium produces the various crithidia and trypanosome types of organism seen in the development of the trypanosome in *Triatoma megista.* Animals may be infected with the cultural forms. In the writer's experience, it is very difficult to obtain subcultures. Noguchi (1924*a*), working at yellow fever in Brazil, on one occasion cultivated from a patient's blood, not only the leptospira of yellow fever, but also a trypanosome, the presence of which had not been suspected. The trypanosome, which was probably *T. cruzi,* remained alive in the leptospira medium for many weeks. No statement regarding subculture was made.

Susceptibility of Animals.—*T. cruzi* is readily inoculable into laboratory animals, though there is a marked tendency for it to change its virulence. Guinea-pigs infected by inoculation of the intestinal contents of the bug, *Triatoma megista,* frequently die in a couple of weeks. On the other hand, passage through guinea-pigs for some time may lead to such a decrease of virulence that the animals only acquire a temporary infection, from which they recover. For this reason a strain is best kept up by changing the animal host from time to time. Mice, rats, rabbits, dogs, and cats can all be infected, as also monkeys (*Macacus* and *Cercopithecus*) and marmosets. As will be seen below, the armadillo also acquires an infection. The virulence of the strain may be so low that it can be kept only in very young animals, which are more susceptible than older ones.

Transmission.—As already stated above, Chagas (1909) first showed that *Trypanosoma cruzi* could be transmitted to animals by allowing infected bugs (*Triatoma megista*) to feed on them (Fig. 208), an observation which he later (1912) extended to two other species (*T. infestans* and *T. sordida*). Larvæ hatched in the laboratory became infective in ten to twenty-five days after feeding on infected animals, and this, according to Chagas, was associated with the appearance of small trypanosomes in the body cavity fluid and in the salivary glands. The details of the development are, however, not as well understood as that of *Trypanosoma gambiense* in *Glossina palpalis.* Working with imported bugs in France, Brumpt (1912) found that the trypanosomes of the blood type ingested by the larvæ quickly became changed into stumpy crithidia forms, which reproduce rapidly (Fig. 209, 1-8). The daughter individuals become elongated, and transform themselves into flagellates of the long crithidia type, till the posterior part of the mid-gut contains large numbers of these forms in varying stages of division (Fig. 209, 9-12). After about twenty days amongst the multiplying crithidia forms, there appear smaller trypanosome forms which have been evolved from the former by migration of the kinetoplast towards the posterior end (Fig. 209, 13-16). As the larvæ become older, the small metacyclic trypanosomes appear to be the

dominant type present in the intestinal infection, which was still found to persist five months after the feed on infected blood. The fæces of the infected bug contain numerous metacyclic trypanosomes, and are infective to animals (Fig. 205). The infectivity of the fæces commences with the appearance of the trypanosome forms. Chagas (1909), as noted above, described flagellates of the trypanosome type in the body cavity fluid of the bugs, and stated that he had also seen them in the smears of the salivary glands. This infection is supposed to spread from the gut by way of the Malpighian tubes. According to Brumpt, this phase cannot be of constant occurrence, as he was unable to demonstrate it, even when he examined bugs with a heavy intestinal infection. Torres (1915) failed to demonstrate flagellates in the body cavity fluid of infected reduviids (*T. megista*), though he succeeded in infecting animals by allowing the bugs to bite through gauze, which prevented fæcal contamination of the skin. Extending his observations, Brumpt (1912) was able to demonstrate that, in addition to *T. megista*, other allied species are easily infected—*T. infestans*, *T. chagasi*, and *T. sordida*—while Brumpt and Gonzales-Lugo (1913) proved this for *Rhodnius prolixus*, another reduviid. Brumpt also obtained development in the bed bugs, *Cimex lectularius*, *C. rotundatus*, and *C. boueti*, and even in the tick, *Ornithodorus moubata*. Mayer and Rocha Lima (1914) found that infection of the intestine of *T. megista*, which was associated with a penetration of the epithelial cells by the trypanosomes, persisted for at least two years. They also showed that *T. cruzi* would undergo development in the bed bug, *C. lectularius*, and the

FIG. 208.—*Triatoma megista* (♀), ONE OF THE TRANSMITTING HOSTS OF *Trypanosoma cruzi* (× 3). (AFTER CHAGAS, 1909.)

Dorsal view and side view of head, showing recurved proboscis.

tick, *O. moubata*, but that infection was not transmitted by their bites. Mayer (1918) found that specimens of *O. moubata* still contained infective flagellates in the intestine five years after feeding on an infected animal.

Neiva (1913*a*), experimenting on the transmission of canine piroplasmosis by means of *Rhipicephalus sanguineus*, infected dogs with *Trypanosoma cruzi*. In the bed bug Brumpt found that the infection persisted for over two months, and that the same forms occurred as in the true host, *Triatoma megista*. Moreover, the fæces of the bed bug were infective to animals. In one instance, Blacklock (1914) was able to transmit *T. cruzi* by allowing infected *Cimex lectularius* to feed on an animal. Yamasaki (1924) experimented with the dog flea, but found that in this insect a rapid degeneration of the trypanosomes took place. Brumpt (1914)

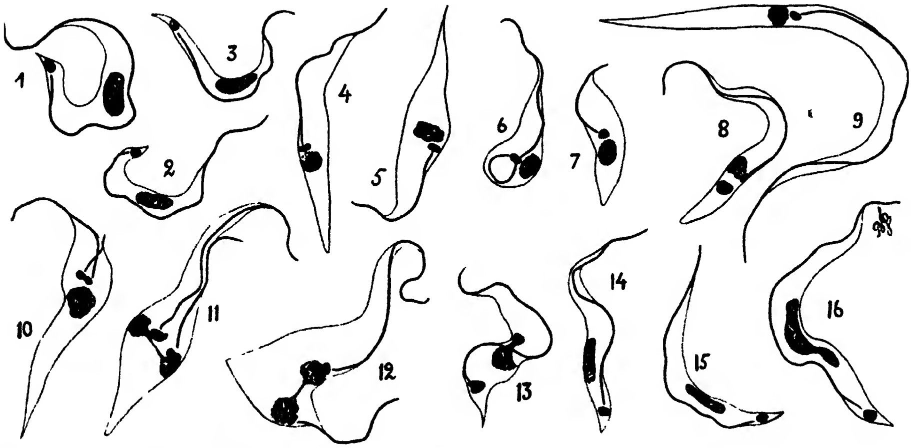


FIG. 209.—DEVELOPMENT OF *Trypanosoma cruzi* IN GUT OF *Rhodnius prolixus* (×2,000) FROM A FILM OF THE INTESTINAL CONTENTS. (ORIGINAL FROM PREPARATION MADE BY DR. TEJERA).

1-3. Trypanosomes of the blood type which are ingested by the bug.
4-9. Various crithidia forms. 10-12. Dividing crithidia forms.
13-16. Metacyclic trypanosomes which escape in the fæces of the bug.

noted that reduviid bugs had the habit of attacking each other, and also of ingesting the liquid fæces passed by themselves or other bugs. That infection may be acquired in this manner was proved by feeding bed bugs on diluted fæces containing crithidia forms of *T. cruzi*. Some of the bugs became infected, and the flagellates persisted in them for over two months. Though admitting the cannibalistic habits of the reduviid bugs, Torres (1915) does not think they can infect one another, as they only suck the clear body cavity fluid, in which he could find no evidence of trypanosomes. Though he found that bugs feed on one another's fæces when in captivity, he believes that under natural conditions this does not occur.

Hoffmann (1922) again calls attention to this habit in the case of *Rhodnius prolixus*. The larvæ were able to continue their development by sucking blood from the recently fed parent bugs or other larvæ. The possibility of their becoming infected in this way is evident.

In Venezuela, Tejera (1919*b*) found naturally infected with *T. cruzi*, not only *R. prolixus*, which is the natural vector, but also another reduviid bug, which he informs the writer has been since identified as *Erathyrus cuspidatus*. Neiva and Pinto, quoted by Pinto (1923, 1924), have effected transmission by means of *R. pictipes*.

It will be seen from the above account that active development of *T. cruzi* takes place in the mid- and hind-gut of the reduviid bugs, and that crithidia and finally metacyclic trypanosome forms appear in the fæces. Chagas believes that the latter gain access to the salivary glands, and that the bugs produce infection by their bites. In most cases, however, this salivary gland infection does not take place, and as the fæces of the bugs are infective when injected into animals, natural infection may occur by the wound inflicted by the bug becoming contaminated with fæces passed by the bug while feeding or by the fæces being ingested, as in the case of *T. lewisi*. Brumpt (1913*a*) and Mayer and Rocha Lima (1914) have shown that mice may be infected by placing infective blood on the buccal mucous membrane, so that oral infection by means of fæces of an infected bug may occur. Brumpt (1912) showed that *T. cruzi* could penetrate the healthy conjuctiva, and subsequently (1913*a*) showed that infection could take place through the healthy skin of young mice.

Reduviid bugs are found naturally infected, not only in the districts in which the human disease is endemic, but also in other localities. Thus, Neiva (1914) in the State of Rio noted that *Triatoma vitticeps*, and in the State of San Salvador, *T. sanguisuga*, *T. dimidiata*, and *R. prolixus*, might be infected with *Trypanosoma cruzi*, while Maggio and Rosenbusch (1915) described the infection of *T. infestans* in the Argentine. Brumpt and Gomes (1914) have found *T. chagasi* naturally infected far from human habitations. This seems to suggest that the bug infection is dependent on some other host than man, in whom infection occurs only in certain localities. Pinto (1923) states that *Triatoma brasiliensis* has been found infected in various parts of Brazil, and that dogs may be infected with the trypanosomes they harbour. A natural infection with *T. cruzi* has been demonstrated in the following reduvid bugs: *Triatoma megista*, *T. infestans*, *T. sordida*, *T. dimidiata*, *T. chagasi*, *T. geniculata*, *T. vitticeps*, *T. sanguisuga*, *R. prolixus*, and *R. pictipes*, though they have not all been incriminated as transmitting the disease to man.

Reservoir Hosts.—Chagas (1912) noted that *T. geniculata* harboured a flagellate in its intestine which was indistinguishable from the develop-

mental forms of *T. cruzi* in the intestine of *Triatoma megista*. *T. geniculata* lives in the burrows of the armadillo (*Dasypus novemcinctus*), which is commonly infected with a trypanosome. Both this trypanosome and the flagellate of the bug were inoculable to guinea-pigs. The trypanosome which appeared in each case resembled *T. cruzi*, and Chagas concluded that the armadillo was a reservoir host. Torres (1915) showed that in the endemic centres of the disease three species of armadillo (*D. novemcinctus*, *D. sexcinctus*, and *D. unicinctus*) were often naturally infected with *T. cruzi*. In the burrows in which these animals lived, a reduviid bug, *Triatoma geniculata*, fed upon them. Chagas (1918) found that as many as 46 to 50 per cent. of armadillos (*D. novemcinctus*) harboured the trypanosome, as did also the bugs, *T. megista*, living in their burrows. The armadillos were frequently found infected far from human habitations. It would appear, therefore, that the armadillo is the natural host of a trypanosome which occasionally infects man. Crowell (1923) examined the organs of a naturally infected armadillo captured in Brazil, and found the usual developmental form of *T. cruzi* in the muscle fibres of the heart. A cat was found by Chagas (1909) to be naturally infected. Chagas (1924) has found that in Brazil in the Para district monkeys (*Chrysothrix sciureus*) may be naturally infected with *T. cruzi*. The trypanosome was inoculable to guinea-pigs and young dogs. The latter animals died of the infection, and were found to have the characteristic reproduction forms in the heart muscles.

OTHER FLAGELLATES RELATED TO TRYPANOSOMA CRUZI.

It is probable that certain flagellates which have been found in the gut of blood-sucking reduviid bugs are closely related to *T. cruzi*. Thus, Lafont (1912) described a form seen by him in the gut of *Triatoma rubrofasciata* in Mauritius. In the gut of the bug there occurred trypanosome, crithidia, and leishmania forms, which resemble very closely the stages of *T. cruzi* in its invertebrate host. Encysted leishmania forms were also described as occurring in the rectum, but from the figures given there is no evidence that a cyst wall actually exists. It is of interest to note that Lafont was able to infect mice by intraperitoneal injection of the gut contents of the bug. Trypanosomes appeared in the blood of the mice, and remained there up to a maximum of eight days. A transitory infection was also produced in the rat and the monkey (*Macacus cynomolgus*). It seems probable that this flagellate, which Lafont named *T. boylei*, will be found to be a trypanosome of some vertebrate. *Crithidia conorhinæ*, described by Donovan (1909*a*) from *Triatoma rubrofasciata* in India, is also possibly a vertebrate trypanosome. The same

remark applies to *T. triatomæ*, described by Kofoid and McCulloch (1916) from the bug, *Triatoma protracta*, which lives in the nest of the wood rat, *Neotoma fuscipes*, of California. *Herpetomonas rangeli* Tejera, 1920, from *Rhodnius prolixus*, and *Crithidia vacuolata* Rodhain, Pons, Vandenbranden, and Bequært, 1913, from *Rhinocoris albopilosus*, may also represent the invertebrate phases of trypanosomes.

III. NON-PATHOGENIC TRYPANOSOMES TRANSMITTED BY SPECIES OF TABANUS, MELOPHAGUS, OR OTHER BLOOD-SUCKING ARTHROPODA.

Trypanosomes of Cattle.

Trypanosoma theileri Laveran, 1902.—Synonyms: *T. transvaaliense* Laveran, 1902; *T. lingardi* Blanchard, 1904; *T. himalayanum* Lingard, 1906; *T. indicum* Lingard, 1907; *T. muktesari* Lingard, 1907; *Trypanozoon theileri* Lühe, 1906; *Trypanosoma wrublewskii* Wladimiroff and Yakimoff, 1908; *T. americanum* Crawley, 1909; *T. frank* Frosch, 1909; *T. falshawi* Knuth, 1909; *T. scheini* Knuth, 1909; *T. rutherfordi* Hadwen, 1912; *T. schönebecki* Mayer, 1913.

Theiler (1903) described a large trypanosome which he had found in cattle in South Africa. He had sent blood-films to Laveran, who (1902*a*) named it *T. theileri*. Since that date similar forms have been discovered in various parts of the world, and have received different names. Lingard (1903-1907) described three species from Indian cattle—*T. himalayanum*, *T. indicum*, and *T. muktesari*. Frosch (1909) described as *T. frank* a trypanosome of cattle in Germany, while Knuth (1909) recorded *T. falshawi* and *T. scheini* from Singapore and Annam. Watson and Hadwen (1912) saw a similar form, named *T. rutherfordi*, in Canada. Crawley (1909) gave the name *T. americanum* to a trypanosome of American cattle. A large form was described from the Lithuanian bison by Wrublewski (1908), and named *T. wrublewskii* by Wladimiroff and Yakimoff (1908). The last observer (1915) came to the conclusion that the trypanosome was in reality *T. theileri*. *T. transvaaliense* was described by Laveran (1902*a*) from blood-films from South African cattle sent him by Theiler. He regarded it as a distinct species, because the kinetoplast was midway between the nucleus and posterior end of the body instead of being near the posterior end. For the same reason Croveri (1920) suggested that the form in cattle in Somaliland was a variety, *T. theileri* var. *somalensis*. It is now known that this degree of variation in the position of the kinetoplast occurs in *T. theileri*.

T. theileri is a large trypanosome measuring 60 to 70 microns in length and 4 to 5 microns in breadth, and frequently shows well-marked myonemes (Fig. 210, 3-4, Plate V., M, p. 456). Smaller forms also occur of a minimum length of 25 to 30 microns. It seems reasonable to suppose that the

FIG. 210.—LARGE TRYPANOSOMES OF MAMMALS (×2,000). (1 AND 5, AFTER BRUCE *et al.*, 1915 AND 1910; 2, AFTER KINGHORN AND YORKE, 1913; 3 AND 4, AFTER LÜHE, 1906.)

1. *Trypanosoma cephalophi* of the duiker (*Cephalophus grimmi*).
2. *T. tragelaphi* from the blood of *Tragelaphus spekei*.

3-4. *T. theileri* from the blood of cattle.

5. *T. ingens* from the blood of the reed buck, bush buck, and ox.

forms described under the various names mentioned above belong to this species. The trypanosomes are never very numerous in the blood of adult cattle, and it is highly probable that the variations in size on which the different species are based merely indicate different developmental stages. By injecting the forms which were named *T. transvaaliense* by Laveran into an ox, Theiler, according to Laveran and Mesnil (1912), produced an infection showing the typical *T. theileri* forms. Similar results were obtained by Behn (1910*a*) in Germany. By inoculating calves with the blood of a cow in which trypanosomes had been demonstrated by the cultural method, a comparatively large infection was produced. At first the trypanosomes were small and numerous, but after five days they became scanty and assumed the large form characteristic of *T. theileri*.

T. theileri var. *somalensis*, described from cattle in Somaliland by Croveri (1920), does not differ in any essential respects from *T. theileri*. It is commonly seen in animals used for the preparation of rinderpest serum, and is said to become pathogenic during the course of this disease.

T. theileri has frequently been demonstrated in the blood of cattle by the cultural method when direct blood examination has been negative. The first experience of this kind was that of Miyajima (1907), who was attempting to cultivate a cattle piroplasm in Japan. In the cultures flagellates appeared, and he supposed he had demonstrated a flagellate stage in the development of the piroplasm. Miyajima's experiments were repeated by Martini (1909) in the Philippines. He was able to demonstrate that the flagellates had no connection with the piroplasm. These results were confirmed by various observers in Europe, Africa, and America, and it was shown that the flagellates in the cultures were derived from *T. theileri*, which was present in very small numbers in the blood. The culture is made by abstracting sterile blood from the jugular vein, and adding it to twice its volume of ordinary nutrient bouillon. The mixture is kept at a temperature of about 25° C., and flagellates of various forms begin to appear towards the end of a week, and attain their maximum in a fortnight. Subculture may be carried out in the same medium or in blood-agar media. In the cultures every variety of form between small round bodies of the leishmania type having a diameter of 2 to 3 microns up to large crithidia forms occur. The largest forms which may have the trypanosome structure are 60 to 70 microns in length, and resemble *T. theileri* as seen in the blood. Herds of cattle examined by the culture method have shown a percentage of infected individuals varying from 10 to 70 per cent. As far as is known, the infections in no way inconvenience the host.

Theiler (1903) claims to have transmitted the trypanosome through the agency of *Hippobosca rufipes* and *H. maculata*. Flies fed on infected

cattle were at once transferred to uninfected animals, and in two cases out of four an infection resulted. Such a transmission, if it actually took place, is evidently a purely mechanical one, which might be accomplished by any biting insect. The difficulty of excluding an infection in the cattle apart from the culture method, which was not employed by Theiler, raises doubt as to whether the experimental animals were really free from infection before exposure to the flies.

Nöller (1916) succeeded in obtaining a culture on blood-agar of *Crithidia subulata*, a flagellate first described by Leger, L. (1904*c*), from the gut of *Tabanus glaucopis*, and, owing to the resemblance of the cultural forms to those of *T. theileri*, he came to the conclusion that *C. subulata* is

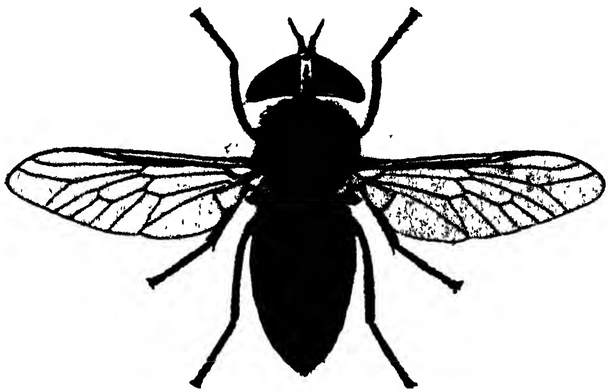


FIG. 211.—*Tabanus tæniola* (*T. socius*) (♀) OF THE SUDAN, WITH WINGS EXTENDED (×2·5). (AFTER KING, 1911.)

This species very commonly harbours a crithidia, which is probably a developmental form of *Trypanosoma theileri*.

really the developmental form of *T. theileri* in the tabanid fly, which is to be regarded as the true insect host of this trypanosome. It has been suggested above (p. 358) that *C. hyalommæ*, which occurs in the tick, may possibly be a developmental form of this trypanosome. If *C. subulata* is merely the insect phase of *T. theileri*, it seems probable that this applies also to other similar flagellates of Tabanidæ and their allies, such as those seen by the writer (1909) in the Sudan. They were especially common in *Tabanus tæniola* (*T. socius*), which was a voracious blood-sucker (Fig. 211). Nöller (1925) appears to have established this identity in the case of the crithidia of *Hæmatopota pluvialis*. He injected clean calves with cultures of the flagellate of the flies and recovered trypanosomes from the blood by culture on the fifth, sixth, and tenth days.

Trypanosomes of Sheep.

Trypanosoma melophagium (Flu, 1908).—Synonyms: *Crithidia melophagia* Flu, 1908; *Leptomonas* Roubaud, 1909; *L. melophagi* Mesnil, 1909; *C. melophagi* Swingle, 1909; Sheep-trypanosome Woodcock, 1910; *Crithidia* Wenyon, 1913; *L. melophagia* Brumpt, 1913; *T. woodcocki* Brumpt, 1913; *Herpetomonas melophagia* Doflein, 1916; *Trypanosoma (Cystotrypanosoma) melophagia* Brumpt, 1922.

This trypanosome, the developmental stages of which in the sheep ked (*Melophagus ovinus*) were the first forms to be discovered, was seen by Pfeiffer (1905), who referred to it as a "trypanosome-like flagellate." Flu (1908) described the ked flagellate as *Crithidia melophagia*, and, like its original discoverer and many subsequent observers, including Roubaud (1909), Porter (1910), Swingle (1911*a*), Dunkerley (1913), regarded it as an

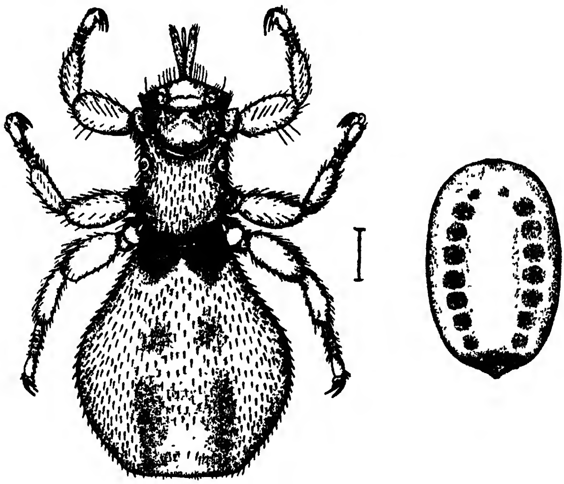


FIG. 212.—THE SHEEP KED, *Melophagus ovinus* (♀), AND ITS PUPA, THE TRANSMITTER OF *Trypanosoma melophagium* (× 8). (AFTER HOARE, 1923.)
The scale shows the natural size of the fly.

organism peculiar to the ked. Woodcock (1910), however, observed a trypanosome in the blood of an English sheep, and suggested the possibility of the ked flagellate being merely the invertebrate phase of this parasite. The trypanosome of sheep was again seen by Behn (1911, 1912) in Germany, and its relation to the ked flagellate was investigated by Nöller (1917) and Kleine (1919*a*). Nöller obtained cultures of both the sheep trypanosome and the ked flagellate, and showed that the cultural forms were identical. He noted that flocks of sheep which were most heavily infested with keds were likewise most heavily infected with trypanosomes, and he concluded that the ked flagellate was actually the developmental form of

the sheep trypanosome, as Woodcock had suggested. Nöller pointed out that its correct name was *T. melophagium.* Kleine (1919*a*) also studied the trypanosome, and came to the conclusion that the ked inoculated it to sheep from its salivary glands. The whole question has been the subject

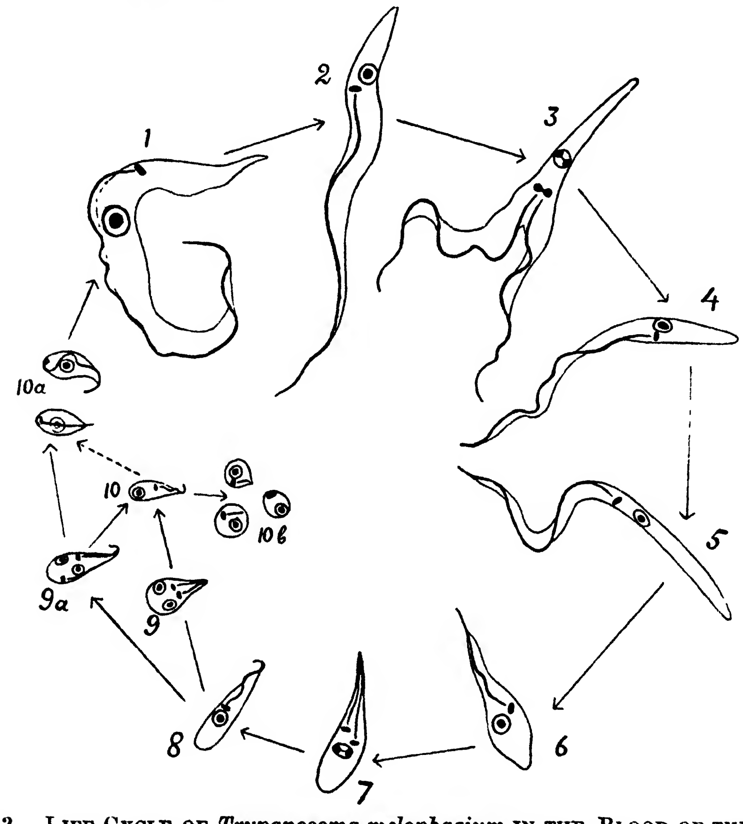


FIG. 213.—LIFE-CYCLE OF *Trypanosoma melophagium* IN THE BLOOD OF THE SHEEP AND IN THE KED (*Melophagus ovinus*) (× 1,560). (AFTER HOARE, 1923; FROM *Parasitology*, vol. xv., p. 395.)

1. Trypanosome in blood of sheep; form ingested by ked.
2. Trypanomorphic crithidia form which leads to typical crithidia (4, 5) by division (3).
3. Dividing form. 4-5. Typical crithidia forms in mid-gut.
6-8. Development of small crithidia forms in hind-gut.
9 9*a*. Two methods of division of crithidia forms, giving rise either to small pyriform crithidia (10) or metacyclic trypanosomes (10*a*). By migration of the kinetoplast the crithidia may become a metacyclic trypanosome (10, 10*a*).
10*b*. Leishmania forms taking no part in cycle.

of exhaustive investigation by Hoare (1922, 1923) in England. He has shown conclusively that uninfected lambs can be infected by feeding them with the hind-gut of infected keds, and, furthermore, that the bite of the ked is unable to bring about infection. A study of the trypanosome in the ked has shown that the flagellate produces metacyclic trypanosomes in

the hind-gut, and that the development is one in the posterior station, as in the case of *T. lewisi* in the flea (Fig. 213). The many observers who regarded the ked flagellate as peculiar to the insect have described encysted forms in the rectum, and it was supposed that these were ingested by other keds, which consequently became infected. That such an infection did not take place was proved by Kleine (1919*a*), who found that uninfected keds hatched from pupæ in the laboratory did not become infected when kept with keds already infected. He showed, furthermore, that uninfected keds did not become infected when fed on goats which did not harbour trypanosomes. It is evident, therefore, that the bodies described as cysts in the fæces of the keds by various observers who have investigated

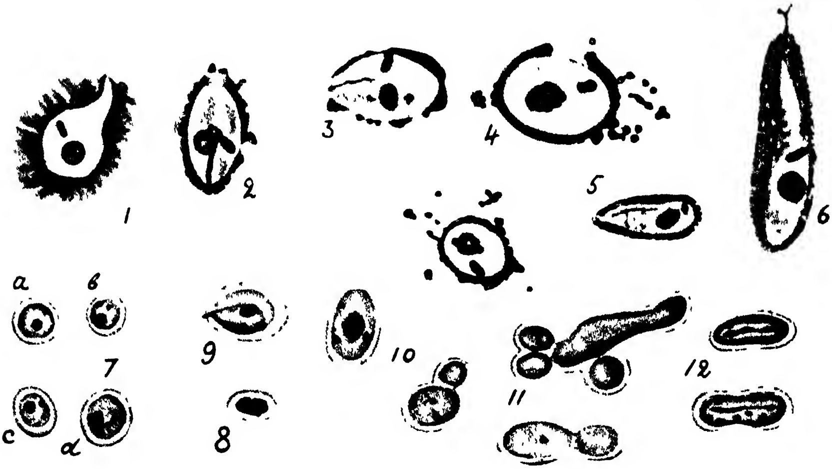


FIG. 214.—STRUCTURES IN THE HIND-GUT OF THE KED, WHICH MIGHT BE INTERPRETED AS CYSTS OF FLAGELLATES (×2,000). (AFTER HOARE, 1923.)

1. Accumulation of staining material round a flagellate producing appearance of a homogeneous cyst wall. 2-5. Stained granular débris round leishmania forms. 6. Deposit round short flagellate form. 7-8. Yeasts of the *Cryptococcus* type. 9. Metacyclic trypanosome superimposed on a yeast. 10-12. Yeasts in various stages.

this flagellate were not of this nature. They were in many cases leishmania forms round which deposits of stain had taken place, or even other organisms, such as yeasts (Fig. 214). It is possible that the cysts which have been described in the case of *H. grayi* of tsetse flies may be of a similar nature. The cycle of development of the ked flagellate, as described by Porter (1910), in which the various phases (pre-flagellate, flagellate, and post-flagellate) occur are quite erroneous. The work of Hoare has finally established the identity of the ked flagellate and the trypanosome of sheep, and, furthermore, shows that many of the *Crithidia* of blood-sucking arthropods require reinvestigation from the point of view of their possible relationship to vertebrate trypanosomes. *T. melophagium*

is usually present in small numbers in the blood of infected sheep, and, as in the case of *T. theileri*, its presence is best detected, as first shown by Behn (1911), by the use of thick films or, as Nöller (1920*c*) demonstrated, by abstracting blood from a vein and diluting it in culture tubes under sterile conditions with an equal quantity of bouillon. The mixture is incubated at 30° C. for a week or more, after which time the scanty trypanosomes will have multiplied sufficiently to be readily detected. By the culture method Hoare was able to demonstrate that the sheep in a ked-infested flock were infected to the extent of 80 per cent. In lambs which were experimentally infected by feeding them with the hind-gut of keds, the trypanosomes are for a short time sufficiently numerous to be detected in the blood by the examination of a few wet films. The infection, however, subsides in the course of one to three months, and if the animals are kept free from keds it will disappear entirely. The sheep, however, can be readily reinfected, and it seems probable that there is only a very slight degree of immunity, and that flocks of sheep are kept infected by constant reinfection. The trypanosome appears to have no harmful effect on the sheep.

The trypanosome in the blood of the sheep is of large size, like *T. theileri* (Fig. 215). It is from 50 to 60 microns in length, and the portion of the body behind the kinetoplast is pointed and represents about one-third the length of the entire body. The nucleus is central in position, and the kinetoplast is a short distance behind it and about 9·6 microns from the posterior end. There is a short free flagellum about 5·6 microns in length. No multiplication forms have been seen in the blood.

The early stages of development in the ked have not been followed, but these insects are practically invariably infected when taken off sheep. The predominating type is a crithidia which appears to be confined to the stomach (Fig. 215, 2-3). It multiplies rapidly by longitudinal fission, and becomes attached in large numbers to the wall of the hind-gut, especially round the pyloric opening of the stomach. In this attached condition many of the crithidia forms by repeated divisions unassociated with growth become smaller forms, which by migration backwards of the kinetoplast to the posterior extremity of the body are transferred into short stumpy metacyclic trypanosomes (Fig. 215, 4-5). The latter are presumably those which lead to infection of the sheep. They resemble in many respects the small metacyclic trypanosomes of *T. lewisi*.

Cultures of the trypanosome, whether commenced from the blood of sheep or from the intestine of the ked, can be maintained at 30° C. in Nöller's medium, which consists of N.N.N. medium to which glucose has been added. In the cultures from the sheep's blood large trypanosomes at first occur, but these quickly become crithidia forms like those in cultures

from the ked's gut. In older cultures of both kinds there appear numbers of small trypanosomes, which are like the small metacyclic forms developed in the hind-gut of the ked. In fact, the behaviour of the trypanosome in cultures appears to be directly comparable with its development in the ked.

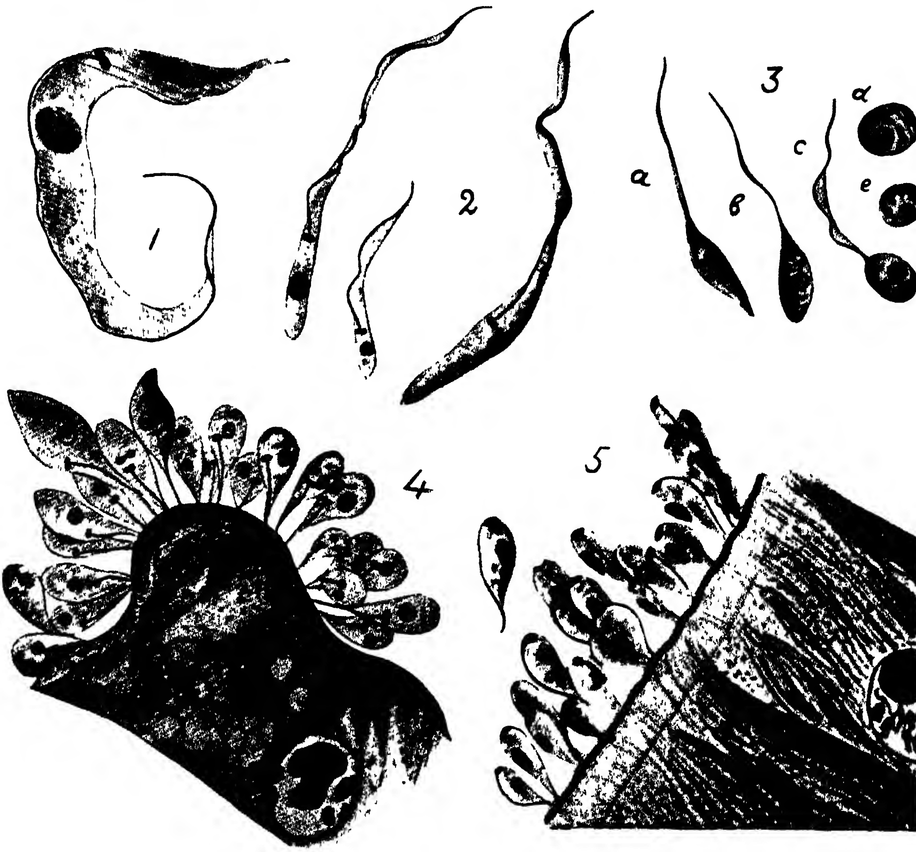


FIG. 215.—*Trypanosoma melophagium* OF THE SHEEP AND SHEEP KED, *Melophagus ovinus* (×2,000). (AFTER HOARE, 1923.)

1. Trypanosome from blood of sheep.
2. Three crithidia forms from mid-gut of ked.
3. Small crithidia and leishmania forms from mid-gut of ked.
4. Epithelium of hind-gut of ked with various attached flagellates.
5. Metacyclic trypanosomes attached to epithelium of hind-gut.

Attempts to inoculate mice, rats, and guinea-pigs with the flagellates from the ked and with cultures have been invariably unsuccessful except in the case of Laveran and Franchini (1914, 1919), who claim to have infected mice by feeding them or inoculating them intraperitoneally with the flagellates from the ked. The infection, however, was said to be

of the leishmania type. Galli-Valerio (1923) claims to have produced a similar infection in a rat. If these results are accurate, this is the only known instance of a trypanosome producing a leishmania infection without the occurrence of trypanosomes at the same time. Hoare (1921*a*) in the case of rats, mice, and guinea-pigs, and Buchner (1922) with mice, failed entirely to produce any infection with these flagellates.

Examining ticks (*Ixodes ricinus*) from sheep, Bishop (1911) claims to have seen a single crithidia form in the tick. It is possible this was a cultural form of the sheep trypanosome.

Trypanosomes of Antelope.

Dutton, Todd, and Tobey (1906) described as *T. tragelaphi* a large trypanosome from the blood of a West African bush buck, *Tragelaphus sylvaticus* (Fig. 210, 2). Kleine and Fischer (1911) found a similar form in the reed buck, *Cervicapra arundinum*, near Tanganyika, and Rodhain, Pons, Vandenbranden, and Bequært (1913*a*) one in *Cephalopus grimmi* and *Cobus vardoni* in the Congo. It does not seem improbable that these forms are actually *Trypanosoma theileri*. Bruce, Hamerton, Bateman, and Mackie (1909*a*) discovered a much larger form in the reed buck (*Cervicapra arundinum*), in the bush buck (*Tragelaphus sylvaticus*), and in an ox in Uganda. On account of its large size it was named *Trypanosoma ingens* (Fig. 210, 5). It measures from 72 to 122 microns in length and 7 to 10 microns in breadth. The trypanosome was also seen by Fraser and Duke (1912*a*) in the blood of a bush buck in Uganda. From the dimensions given, it will be seen that it is distinctly larger than any known form of *T. theileri*, and on this account is possibly a distinct species. Nothing is known of its life-cycle. A trypanosome of the same type was seen by Dodd (1912) in the blood of two mouse deer (*Tragulus javanicus*) which had died in the Zoological Gardens of Sydney. Bruce *et al.* (1913*c*) gave the name *Trypanosoma cephalophi* to a large form seen by them in the blood of the duiker, *Cephalophus grimmi* (Fig. 210, 1).

Group B. Trypanosomes which Develop in the Anterior Station in the Invertebrate or have become Secondarily Adapted to Direct Passage from Vertebrate to Vertebrate.

1. PATHOGENIC TRYPANOSOMES TRANSMITTED BY BLOOD-SUCKING ARTHROPODA.

General Remarks on the Pathogenic Trypanosomes.

Under this heading are included certain trypanosomes which produce disease in man and domestic animals. As stated above, the true vertebrate hosts of these trypanosomes, in tsetse fly areas of Africa at least,

are not those in which disease is produced, but rather the wild animals of the country, which harbour them without suffering in any serious manner, just as *Trypanosoma lewisi* occurs in the rat. In other parts of the world, with the exception of South America, where the capibara is said to be the reservoir for *T. equinum*, the pathogenic trypanosomes, which are of the *T. evansi* type, appear to be transmitted amongst the domestic animals alone. This is undoubtedly accounted for by the fact that it is only in Africa that domestic animals come into close contact with the game. It is on account of the importance of these trypanosomes from an economic standpoint that they have attracted so much attention.

In tsetse-fly areas of Africa the domestic animals have been found infected as follows:

HORSE, MULE, and DONKEY: *T. brucei*, *T. vivax*, *T. congolense*.

OX: *T. gambiense* (?), *T. brucei*, *T. vivax*, *T. congolense*, *T. uniforme*, *T. montgomeryi*.

PIG and CAMEL: *T. brucei*, *T. congolense*.

SHEEP and GOAT: *T. gambiense* (?), *T. brucei*, *T. vivax*, *T. congolense*, *T. capræ*.

DOG: *T. gambiense*, *T. brucei*, *T. congolense*, *T. montgomeryi*.

Relation to Game.—In Nyasaland in the fly country below Kasu Hill, the Royal Society's Commission under Bruce (1913*e*) found that the wild game harboured trypanosomes to the extent of 31·7 per cent. The species found were *T. brucei* (7·8 per cent.), *T. pecorum* (*T. congolense*) (14·4 per cent.), *T. simiæ* (1·7 per cent.), *T. capræ* (11·1 per cent.), and *T. ingens* (1·7 per cent.). As regards the wild tsetse flies (*Glossina morsitans*), of 1,060 examined by Bruce *et al.* (1914*f*) *T. brucei* was found once, *T. pecorum* six times, *T. simiæ* twelve times, and *T. capræ* fourteen times. Similar results had previously been obtained by Bruce (1895) in Zululand, though at that time all the pathogenic trypanosomes were considered to belong to the species *T. brucei*.

Domestic animals living in the area were found infected to a limited extent, but their numbers were so small as to constitute little danger. Of 140 goats examined, five showed *T. pecorum* and one *T. capræ*; and of twenty-two dogs, six harboured *T. pecorum* and ten *T. brucei*.

Kinghorn and Yorke (1912*a*) found that trypanosomes were of frequent occurrence in the domestic stock of North-East Rhodesia. As regards the big game, a conservative estimate placed the percentage of those infected at about 50 per cent. in the Luangwa Valley, and 35 per cent. in the Zambesi-Congo basin. The trypanosomes found were *T. brucei* (*T. rhodesiense*), *T. vivax*, *T. congolense* (*T. nanum* and *T. pecorum*), *T. montgomeryi*, *T. multiforme* (*T. brucei* or *T. gambiense*, or a mixed

infection), and *T. tragelaphi*. The animals harbouring trypanosomes included bush buck, water buck, puku, impala, sitatunga, eland, and duiker. Duke (1913*a*) also found that a considerable percentage of the wild game in West Uganda is infected with trypanosomes (*T. brucei*, *T. congolense*, *T. vivax*, *T. uniforme*, and trypanosomes having a "suspicious resemblance to *T. gambiense*"). Similar results were obtained by Kleine and Fischer (1911), Rodhain, Pons, Vandenbranden, and Bequaert (1912, 1913*a*), Taute (1913), Weck (1914), and others.

The following table given by Bruce and his co-workers (1913*e*) shows the results of the examination of wild animals in Nyasaland:

Animal.	*Number Examined.*	*Number Infected.*	*T. brucei, T. rhodesiense.*	*T. pecorum.*	*T. simiæ.*	*T. capræ.*	*T. ingens.*
Eland	10	6	—	6	—	1	—
Sable	5	0	—	—	—	—*	—
Water buck ..	13	9	3	1	—	8	—
Koodoo	3	2	—	2	—	—	—
Bush buck	10	7	—	7	—	1	—
Hartebeest ..	35	6	5	1	—	—	—
Reed buck	19	12	3	1	—	9	1
Oribi	26	4	1	1	—	1	1
Duiker	7	2	1	—	—	—	1
Buffalo	9	2	—	2	—	—	—
Lion	1	0	—	—	—	—	—
Hyæna	3	2	—	2	—	—	—
Elephant	2	0	—	—	—	—	—
Wart hog	33	7	1	3	3	—	—
Wild cat	3	0	—	—	—	—	—
Porcupine	1	0	—	—	—	—	—
Total	180	59	14	26	3	20	3

The possibility of the existence of a reservoir of *T. gambiense* in game and other animals will be discussed below. The evidence that any such reservoir exists is not at all clear. As regards the other trypanosome of man in Africa, which appears to be merely a strain of *T. brucei*, but which is usually referred to as *T. rhodesiense*, the position is a difficult one. In areas where the disease nagana of domestic animals is common, and the human disease due to this strain of *T. brucei* is absent, all observers are agreed that the trypanosome of this type in the game is *T. brucei*. In areas in which the human disease occurs opinions differ. In Nyasaland the Royal Society's Commission under Bruce (1913 to 1914) concluded that the trypanosome in man, domestic animals, and game was identical, and called it *T. brucei* vel *rhodesiense*. Kinghorn and Yorke (1912 to 1913) in North Rhodesia referred to the trypanosome in man as

T. rhodesiense, and concluded that the similar form in the game was also *T. rhodesiense*. Kleine and Taute, however, in Tanganyika referred to the human form as *T. rhodesiense*, but believed that that which occurred in domestic animals and game was another species—namely, *T. brucei*. According to them, a reservoir host of *T. rhodesiense* has not been discovered. This subject will be referred to in more detail below.

There seems to be little evidence that *T. evansi* (including several named species of trypanosome which appear to be merely races of *T. evansi*), which has a wide distribution in tsetse-free areas of the Old and New World, and which infects cattle, horses, mules, donkeys, camels, and elephants, has any reservoir comparable with the game reservoirs in Central Africa. It has been supposed that the buffalo or pig may act in this capacity in India, while in South America it has been stated that one form (*T. venezuelense*) occurs naturally in the dog, monkey, and capibara, and another (*T. equinum*) in the last-named animal.

Game Reservoirs of Trypanosomes of Men and Domestic Animals in Africa.

BUFFALO (*Bos caffer*): *T. brucei*, Bruce *et al.*, 1897. *T. vivax*, Duke, 1913. *T. uniforme*, Duke, 1913. *T. congolense*, Duke, 1913; Bruce *et al.*, 1913.

BUSH BUCK (*Tragelaphus scriptus*): *T. gambiense* (*T. multiforme*), Kinghorn and Yorke, 1912. *T. brucei*, Bruce *et al.*, 1897; Kleine and Fischer, 1911; Kinghorn and Yorke, 1912; Taute, 1913. *T. capræ*, Bruce *et al.*, 1913. *T. vivax*, Bruce *et al.*, 1911; Kleine and Fischer, 1911. *T. cazalboui* (=*T. vivax*), Rodhain, Pons, Vandenbranden, and Bequært, 1913. *T. uniforme*, Duke, 1912; Fraser and Duke, 1912. *T. congolense*, Kinghorn and Yorke, 1912; Kleine and Eckard, 1913; Rodhain, Pons, Vandenbranden, and Bequært, 1913; Bruce, 1913. *T. dimorphon* (=*T. congolense*), Dutton, Todd and Kinghorn, 1907; Montgomery and Kinghorn, 1908; Johnson, 1920. *T. theileri* (*T. tragelaphi* ?), Dutton, Todd, and Tobey, 1906. *T. ingens*, Bruce *et al.*, 1909; Fraser and Duke, 1912; Rodhain, Pons, Vandenbranden, and Bequært, 1913. *Undetermined*, Montgomery and Kinghorn, 1908; Kleine and Fischer, 1911; Weck, 1914; Dutton, Todd, and Kinghorn, 1907.

CHIMPANZEE: *T. gambiense* [?], Ziemann, 1902.

DUIKER (*Cephalophus grimmi*): *T. brucei*, Bruce *et al.*, 1913; Taute, 1913. *T. vivax*, Kinghorn and Yorke, 1912. *T. congolense*, Kinghorn and Yorke, 1912. *T. theileri*, Rodhain, Pons, Vandenbranden, and Bequært, 1912. *T. ingens*, Bruce *et al.*, 1912; Rodhain, Pons, Vandenbranden, and Bequært, 1912.

ELAND (*Taurotragus oryx*): *T. brucei*, Taute, 1913; Davey, 1916. *T. capræ*, Bruce *et al.*, 1913. *T. congolense*, Kinghorn and Yorke, 1912; Bruce *et al.*, 1913; Davey, 1916. *Undetermined*, Weck, 1914.

ELEPHANT: *T. brucei* (*T. elephantis*), Bruce *et al.*, 1909.

HARTEBEEST (*Bubalis lichtensteini*): *T. brucei*, Kinghorn and Yorke, 1912; Bruce *et al.*, 1913; Taute, 1913. *T. congolense*, Kleine and Fischer, 1911 (Pferdeantelopen); Bruce *et al.*, 1913. *Undetermined*, Montgomery and Kinghorn, 1908.

HIPPOPOTAMUS: *Undetermined*, Kleine and Taute, 1911.

HYÆNA (*Hyæna crocuta*): *T. brucei*, Bruce *et al.*, 1897. *T. congolense*, Bruce, 1913. *Undetermined* (? *T. gambiense*), Duke, 1913.

KOODOO (*Strepsiceros capensis*): *T. brucei*, Bruce *et al.*, 1897. *T. capræ*, Bruce *et al.*, 1914. *T. cazalboui* (= *T. vivax*), Rodhain, Pons, Vandenbranden, and Bequært, 1913. *T. congolense*, Kinghorn and Yorke, 1912; Bruce *et al.*, 1913.

LEMUR (*Galago demidoffi*): *T. gambiensi* (?), Martin, Lebœuf, and Roubaud, 1909.

LION (*Felis leo*): *Undetermined*, Weck, 1914.

MONKEY: *T. gambiense* (?), Kudicke, 1906; Dutton, Todd, and Tobey, 1906; Koch, Beck, and Kleine, 1909; Bruce *et al.*, 1911.

MPALA (*Æpyceros melampus*): *T. brucei*, Kinghorn and Yorke, 1912. *T. capræ*, Bruce *et al.*, 1914. *T. congolense*, Kinghorn and Yorke, 1912; Bruce, 1914.

ORIBI (*Oribia scoparia* ?): *T. brucei*, Bruce, *et al.* 1913. *T. capræ*, Bruce *et al.*, 1913. *T. congolense*, Bruce *et al.*, 1913. *T. ingens*, Bruce *et al.*, 1913.

OTTER (*Lutra capensis* ?): *Undetermined*, Fehlandt, 1911.

PUKU (*Cobus vardoni*): *T. vivax*, Kinghorn and Yorke, 1912. *T. cazalboui* (= *T. vivax*), Rodhain, Pons, Vandenbranden, and Bequært, 1913. *T. ingens*, Rodhain, Pons, Vandenbranden, and Bequært, 1913.

REED BUCK (*Cervicapra arundinum*): *T. gambiense* (? *T. brucei*), Simpson, 1918. *T. brucei*, Bruce *et al.*, 1903 and 1913; Taute, 1913. *T. capræ*, Bruce *et al.*, 1913. *T. vivax*, Connal, 1917; Simpson, 1918. *T. cazalboui* (= *T. vivax*), Rodhain, Pons, Vandenbranden, and Bequært, 1913. *T. congolense*, Kleine and Fischer,* 1911; Bruce *et al.*, 1913. *T. theileri*, Kleine and Fischer, 1911. *T. ingens*, Bruce *et al.*, 1909 and 1913. *Undetermined*, Kleine and Fischer, 1911; Weck, 1914.

ROAN (*Hippotragus equinus*): *T. vivax*, Duke, 1923. *T. cazalboui* (= *T. vivax*), Rodhain, Pons, Vandenbranden, and Bequært, 1913. *T. congolense*, Kinghorn and Yorke, 1912; Davey, 1916.

SABLE (*Hippotragus niger*): *Undetermined*, Weck, 1914.

SERVAL (*Felis serval* ?): *Undetermined*, Weck, 1914.

SITATUNGA (*Tragelaphus spekei*): *T. gambiense*, Duke, 1912. *T. brucei*, Duke, 1921. *T. vivax*, Duke, 1912. *T. uniforme*, Duke, 1912 and 1923. *T. tragelaphi*, Kinghorn and Yorke, 1912; Duke, 1912. *T. ingens*, 1912.

STEINBOCK (*Raphiceros campestris*): *T. brucei*, Bruce *et al.*, 1903.

WART HOG (*Phacoceros æthiopicus*): *T. brucei*, Kinghorn, and Yorke, 1912; Bruce *et al.*, 1913. *T. congolense*, Bruce *et al.*, 1913; Simpson, 1918. *T. simiæ*, Bruce *et al.*, 1913.

WATER BUCK (*Cobus ellipsiprymnus*): *T. brucei*, Kleine and Fischer, 1911; Kinghorn and Yorke, 1912; Bruce *et al.*, 1913; Taute, 1913; Stohr, 1913; Duke, 1923. *T. capræ*, Bruce *et al.*, 1913. *T. vivax*, Kleine and Fischer, 1911; Kinghorn and Yorke, 1912; Duke, 1913; Johnson, 1920. *T. uniforme*, Duke, 1913. *T. congolense*, Kinghorn and Yorke, 1912; Bruce *et al.*, 1913. *T. ingens*, Bruce *et al.*, 1914. *Undetermined*, Kleine and Fischer, 1911; Weck, 1914.

WILDEBEEST (*Connochætes gnu* ?): *T. brucei*, Bruce *et al.*, 1897. *Undetermined*, Weck, 1914.

Mechanism of Infection. — Under natural conditions the pathogenic trypanosomes are transmitted to man and domestic animals by blood-sucking arthropods. In the tsetse-fly areas of Africa those flies which belong to the genus *Glossina* are chiefly responsible, though it is possible that other biting flies may occasionally play a part (Fig. 216). From the table (p. 517) it will be seen that one species of tsetse fly is able to transmit

several species of trypanosome, and this fact led Kleine and Fischer (1912) to express the view that any species of tsetse fly would probably be able to transmit any of the pathogenic trypanosomes with which it was in contact. The flies, which inject the trypanosomes when they bite, become infective after the trypanosome has passed through a definite cycle of development, terminating in the production of metacyclic trypanosomes. The cycle requires about twenty days for its completion. In an ingenious experiment, Rodhain, Pons, Vandenbranden, and Bequært (1912*c*), induced *G. morsitans* infected with *T. brucei* to feed through a membrane covering a tube in which citrated blood was contained. After a fly had fed, the number of trypanosomes in a portion of the fluid were counted, and it was estimated that a single infected fly was able to inject 1,562 metacyclic trypanosomes while feeding.

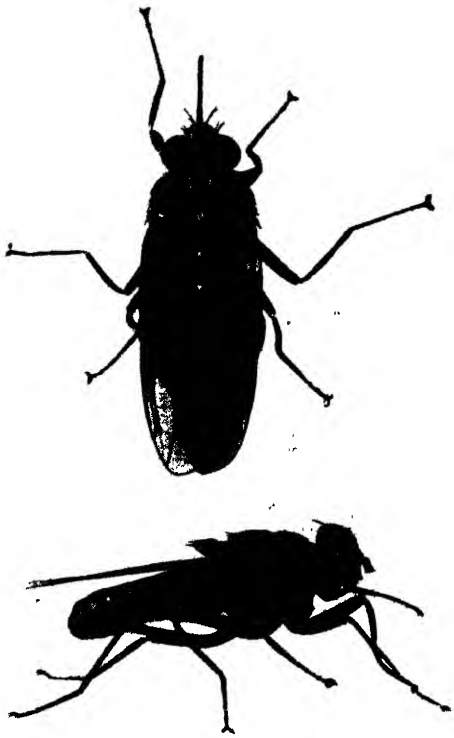


Fig. 216.—*Glossina morsitans* (♀) Dorsal and Side Views (× 4·5). (After Newstead, 1924.)

It has been clearly demonstrated that a purely mechanical transmission may also occur by the fly contaminating the wound it inflicts with infective blood which it has recently taken into its proboscis from another host. Duke (1919) believes that the epidemic of sleeping sickness which swept over Uganda was largely due to mechanical transmission of infection from man to man by *Glossina palpalis*. This view is further developed by Duke (1921, 1923, 1923*a*), who concludes that wherever human trypanosomiasis occurs in epidemic form in Africa, the transmission is a mechanical one. Certain experiments made by him (1923*a*) are held to prove that when the human trypanosome is passed directly from monkey to monkey by direct inoculation of blood, it eventually loses its power of passing through the complete cycle in the tsetse fly, and he assumes that a similar change may occur after prolonged mechanical transmission from man to man.

Hornby (1921) found that mechanical transmission of trypanosomes amongst domestic stock in Rhodesia is by no means uncommon. A few animals which have acquired infection in tsetse-fly areas, if brought into close contact with animals in a tsetse-free district, may lead to the infection spreading through the stock. In such cases infection is spread by flies other than tsetse flies, and presumably in a mechanical manner. All the pathogenic trypanosomes which are transmitted by tsetse flies have been shown by various observers to be capable of mechanical transmission by mosquitoes or species of *Stomoxys* and *Tabanus*. In the case of *T. evansi* and the forms allied to it both in the Old World and America, this is the only method of transmission which has been demonstrated, unless the claim made by Cross and Patel (1921) regarding the transmission of *T. evansi* by ticks in India indicates a cycle of development comparable with that in tsetse flies in Africa. Mechanical transmission of *T. evansi* (*T. hippicum*) by the house fly was proved to be possible by Darling (1912).

Direct inoculation of blood from an infected to a healthy animal will bring about infection, and it is by this means that the various laboratory strains of trypanosomes have been maintained for experimental work. Many strains have been kept in rats or guinea-pigs for numbers of years, but it must always be remembered that such artificially maintained strains may acquire peculiarities which they did not originally possess in the normal host. There is a variation in the animals inoculable with any one trypanosome, and, furthermore, after successive passages the virulence may become much increased. Intraperitoneal and intravenous inoculations lead to infections more readily than subcutaneous ones. It is highly probable that after long maintenance in animals like rats in the unnatural conditions of direct passage, without any fly intervention as occurs in nature, trypanosomes become profoundly altered, not only morphologically, but also physiologically, so that care has to be exercised in comparing such forms with those recently isolated from their natural hosts. Bruce *et al.* (1913*b*) expressed the opinion that "it is absurd to expect to arrive at any classification at all approaching a true one by the study of strains of trypanosomes kept for many years and undergoing many vicissitudes in our European laboratories."

Bruce (1897) noted that a dog which had eaten a piece of the congealed heart blood of a heifer which had died of nagana contracted the disease, while many instances are on record of animals becoming infected after eating the organs of infected animals. Experimental work has demonstrated the infective power of blood introduced into the mouth, stomach, conjunctival sac, and vagina. Under natural conditions it is known that *T. equiperdum* is transmitted through mucous membranes, while rats

become infected with *T. lewisi* by eating the fæces of infected fleas, a method of infection which is probably applicable to other trypanosomes also.

Attempts have been made to infect invertebrates with the pathogenic trypanosome by inoculating blood from infected vertebrates. Wendelstadt and Felmer (1909) proved that *T. brucei* could survive in the tissues

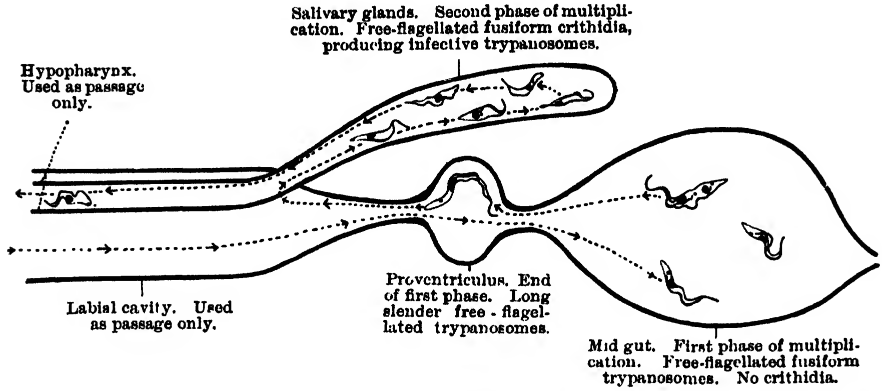


FIG. 217.—DIAGNOSTIC CHARACTERS OF *Trypanosoma brucei* AND *Trypanosoma gambiense* IN THE TSETSE FLY. (AFTER LLOYD AND JOHNSON, 1925.)

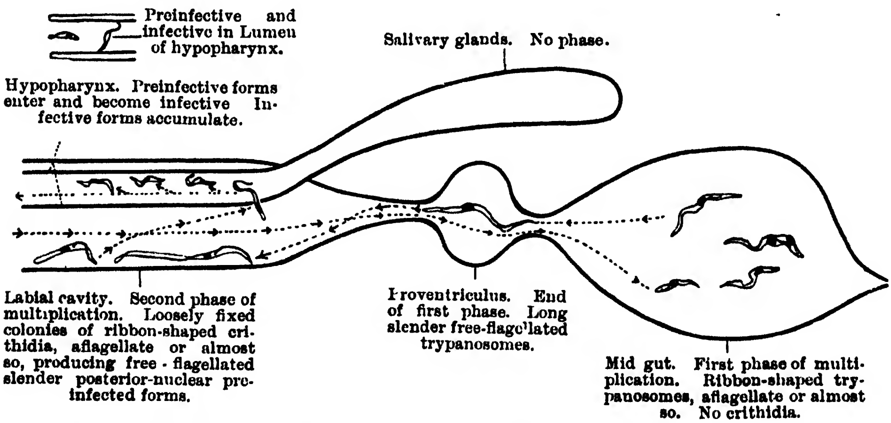


FIG. 218.—DIAGNOSTIC CHARACTERS OF *Trypanosoma congolense* IN THE TSETSE FLY. (AFTER LLOYD AND JOHNSON, 1925.)

of beetles for at least seven days. More recently Iwanow (1925) has found that *T. equiperdum* will live for eleven days in caterpillars (*Galleria mellonella*) kept at laboratory temperature. Active trypanosomes were seen up to the ninth day, while mice could still be infected by inoculation of the tissues of the caterpillars up to the eleventh day.

Identification of Trypanosomes in Tsetse Flies.—As various pathogenic trypanosomes undergo development in tsetse flies, it is of importance to be able to identify them. Most observers have adopted the method of identifying the trypanosomes which appear in animals after the flies have been allowed to feed upon them. This is a laborious method which entails considerable delay. Though it has been possible in many cases to

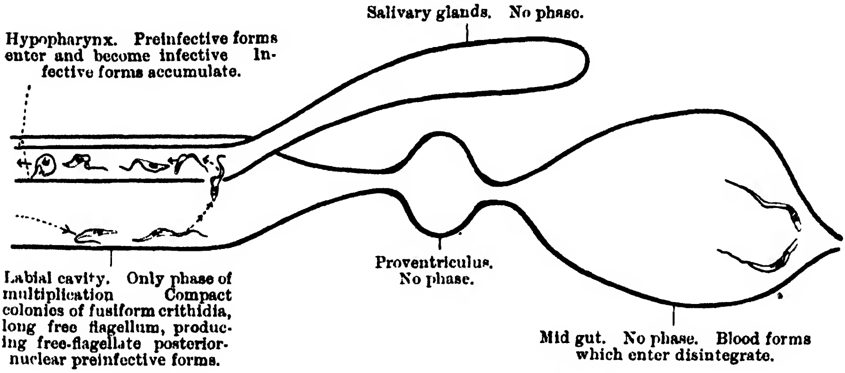


FIG. 219.—DIAGNOSTIC CHARACTERS OF *Trypanosoma vivax* IN THE TSETSE FLY. (AFTER LLOYD AND JOHNSON, 1925.)

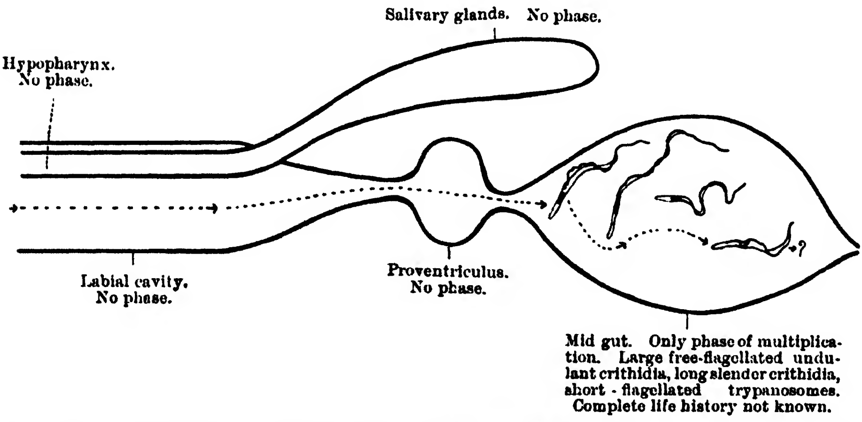


FIG. 220.—DIAGNOSTIC CHARACTERS OF *Trypanosoma grayi* IN THE TSETSE FLY. (AFTER LLOYD AND JOHNSON, 1925.)

make a shrewd guess as to the species from what is known of the site of development in the fly, there has been no certainty about the identification apart from the trypanosomes of the polymorphic type (*T. brucei*, *T. gambiense*), which are known to be the only ones which invade the salivary glands. Lloyd and Johnson (1924), however, after a careful study of the

developmental forms of various trypanosomes in the fly, have reached the conclusion that it is possible to identify the trypanosomes from their morphology alone.

It is known that *T. brucei* and *T. gambiense* develop in the stomach into long thin trypanosomes, which then make their way to the proboscis, enter the hypopharynx, and travel to the salivary glands, where crithidia forms and eventually metacyclic trypanosomes are produced (Figs. 217 and 224). They pass down the hypopharynx with the salivary secretion, and are injected into the vertebrate when the fly feeds. It is evident that in the case of these trypanosomes they may occur in the stomach, proboscis, and salivary glands. Those which may be found in the proboscis are merely travelling forms, either on their way to the salivary glands from the stomach, or from the salivary gland to the vertebrate host. At all stages the trypanosomes have flagella except the metacyclic forms, which resemble the short stumpy trypanosomes occurring in the blood.

In the case of *T. congolense*, development takes place in the stomach, with the production of long slender trypanosomes, which migrate to the proboscis (Figs. 218 and 228). In the labial cavity crithidia forms are produced, and these make their way into the hypopharynx, where the crithidia forms give rise to metacyclic trypanosomes which resemble the blood forms. As in the vertebrate host, all these stages are devoid of flagella, so that they can be distinguished by this character from most of the stages of *T. brucei* and *T. gambiense*. The metacyclic trypanosomes of *T. brucei* and *T. gambiense*, though devoid of flagella, differ from the metacyclic trypanosomes of *T. congolense* in size and other respects.

The development of trypanosomes of the *T. vivax* group in tsetse flies is limited to the proboscis (Figs. 219 and 233). Trypanosomes are taken into the stomach, but these quickly degenerate. Before they do so they can be distinguished from other trypanosomes by their characteristic swollen posterior ends and flagella. The trypanosomes in the proboscis become quickly transformed in the labial cavity into crithidia forms with flagella. These pass into the hypopharynx, where metacyclic trypanosomes of the blood type are evolved. All these stages have flagella.

As regards the trypanosomes which may occur in the stomach, a difficulty is introduced in that another trypanosome, *T. grayi*, commonly occurs in this region (Figs. 220 and 173). It probably represents developmental stages of the trypanosome of the crocodile or the monitor. The characteristic type is a broad crithidia form. Trypanosome forms also occur, but these have very short flagella, and differ in other respects from *T. brucei* and *T. gambiense*. It will thus be seen that the following forms can be recognized in the different regions of the body of tsetse flies in which development occurs:

STOMACH.—*T. brucei* and *T. gambiense:* Trypanosomes of the blood types which multiply and become transformed into long slender trypanosomes with flagella.

T. congolense: Trypanosomes of the blood type which multiply and become transformed into long trypanosomes without flagella.

T. vivax: Trypanosomes of the blood type which quickly degenerate.

T. grayi: Long crithidia forms and long trypanosome forms with very short flagella.

LABIAL CAVITY.—*T. brucei* and *T. gambiense:* Trypanosomes of the blood type on their way to the stomach; long slender trypanosomes with flagella which are passing from the stomach to the hypopharynx.

T. congolense: Trypanosomes of the blood type on their way to the stomach; long trypanosomes without flagella from the stomach; crithidia forms without flagella.

T. vivax: Trypanosomes of the blood type; crithidia forms with flagella.

HYPOPHARYNX.—*T. brucei* and *T. gambiense:* Long trypanosomes with flagella on their way from the stomach and labial cavity to the salivary glands; short stumpy metacyclic trypanosomes without flagella passing from the salivary gland to the vertebrate.

T. congolense: Crithidia forms without flagella from the labial cavity; metacyclic trypanosomes of the blood type.

T. vivax: Crithidia forms from the hypopharynx; metacyclic trypanosomes of the blood type.

SALIVARY GLAND.—*T. brucei* and *T. gambiense:* long slender trypanosomes with flagella from the stomach; crithidia forms; short stumpy metacyclic trypanosomes without flagella.

There seems to be some doubt as to the path taken by trypanosomes in their passage from the labial cavity to the hypopharynx. In the diagrams given by Lloyd and Johnson (Figs. 217-220) they are represented as passing through a slit in the wall of the hypopharynx. Authorities are by no means agreed that such an opening exists. If the hypopharynx, which is really a continuation of the salivary duct, is a closed tube, then it must be supposed that the trypanosomes enter it at its open end at the extremity of the proboscis.

Experimentally Proved Vectors of Pathogenic Trypanosomes of Africa.

GLOSSINA BREVIPALPIS: T. *brucei*, Bruce *et al.*, 1914; Braun and Teichmann, 1914. *T. congolense*, Bruce *et al.*, 1914; Braun and Teichmann, 1914. *T. capræ*, Bruce *et al.*, 1914. *T. simiæ*, Bruce *et al.*, 1914 (dissection of flies only).

GLOSSINA FUSCA: *T. gambiense*, Ross (P. H.), 1908. *Undetermined*, Greig, 1905 (? which fly).

Glossina longipalpis: *T. pecaudi*, Bouet and Roubaud, 1910. *T. dimorphon*, Bouet and Roubaud, 1910. *T. cazalboui*, Bouet and Roubaud, 1910.

Glossina longipennis: *T. dimorphon*, Ross (P. H.), 1913. *Undetermined*, Greig 1905 (? which fly).

Glossina morsitans: *T. gambiense*, Taute, 1911; Rodhain, Pons, Vandenbranden, and Bequært, 1912; Kleine and Fischer, 1912; Bruce, 1915. *T. brucei*, Taute, 1909 (quoted by Kleine); Bruce *et al.*, 1913; Duke, 1916. *T. brucei* (*G. pallidipes* ?), Bruce *et al.*, 1895. *T. brucei* (*T. dimorphon*), Fehlandt, 1911. *T. brucei* (*T. rhodesiense*), Kinghorn and Yorke, 1912; Bruce *et al.*, 1913. *T. pecaudi*, Bouet and Roubaud, 1911; Rodhain, Pons, Vandenbranden, and Bequært, 1912. *T. congolense*, Fehlandt, 1911; Rodhain, Pons, Vandenbranden, and Bequært, 1912; Bruce *et al.*, 1913; Kinghorn and Yorke, 1912; Duke, 1916. *T. dimorphon*, Bouet and Roubaud, 1912. *T. vivax*, Duke, 1916. *T. cazalboui*, Bouet and Roubaud, 1911; Rodhain, Pons, Vandenbranden, and Bequært, 1912; Roubaud, 1915. *T. capræ*, Fehlandt, 1911; Bruce *et al.*, 1913. *T. uniforme*, Duke, 1916. *T. simiæ*, Bruce *et al.*, 1912. *T. simiæ* (*T. ignotum*), Kinghorn and Yorke, 1912.

Glossina pallidipes: *T. gambiense*, Ross (P. H.), 1907. *T. brucei*, Duke, 1916. *T. brucei* (*G. morsitans* ?), Bruce *et al.*, 1895 (?). *T. congolense*, Croveri, 1919. *Undetermined*, Bruce, 1895; Greig, 1905 (? which fly).

Glossina palpalis: *T. gambiense*, Dutton, Todd, and Hannington, 1907; Kleine, 1909; Bruce *et al.*, 1909; Robertson, 1912; Fraser and Duke, 1912; Kleine and Fischer, 1913. *T. brucei*, Minchin, Gray, and Tulloch, 1906; Minchin, 1907; Martin, Lebœuf, and Roubaud, 1908; Kleine, 1909; Fischer, 1911; Fraser and Duke, 1912; Eckard, 1913. *T. pecaudi*, Cazalbou, 1906; Bouet and Roubaud, 1910. *T. congolense*, Bruce, 1910; Fehlandt, 1911; Duke, 1912. *T. dimorphon*, Dutton, Todd, and Hannington, 1907; Roubaud, 1907; Bouet, 1907; Bouet and Roubaud, 1910. *T. vivax*, Bruce *et al.*, 1909. *T. cazalboui*, Bouet, 1907; Bouffard, 1909; Bouet and Roubaud, 1910. *T. uniforme*, Fraser and Duke, 1912. *Undetermined*, Bruce and Nabarro, 1903; Bruce, Nabarro, and Greig, 1903; Nabarro and Greig, 1905; Greig and Gray, 1905.

Glossina swynnertoni: *T. brucei* (*T. rhodesiense*), Duke, 1923.

Glossina tachinoides: *T. gambiense*, Lloyd and Johnson, 1924. *T. brucei*, Lloyd and Johnson, 1924. *T. brucei* (*T. pecaudi*), Macfie, 1914. *T. pecaudi*, Bouet and Roubaud, 1910. *T. congolense*, Macfie, 1914; Lloyd and Johnson, 1924. *T. dimorphon*, Bouet and Roubaud, 1910. *T. vivax*, Macfie, 1914; Lloyd and Johnson, 1924. *T. cazalboui*, Bouffard, 1910; Bouet and Roubaud, 1910.

Stomoxys: *T. gambiense*, Schuberg and Kuhn, 1911. *T. brucei*, Minchin, Gray, and Tulloch, 1906; Martin, Lebœuf, and Roubaud, 1908; Schuberg and Kuhn, 1911. *T. pecaudi*, Bouet and Roubaud, 1912. *T. dimorphon*, Bouet and Roubaud, 1912. *T. cazalboui*, Bouffard, 1907; Bouet and Roubaud, 1912.

Tabanus: *T. brucei*, Sergent (Ed. and Et.), 1906.

Culex: *T. gambiense*, Roubaud and Lafont, 1914. *T. brucei* (*T. rhodesiense*), Roubaud and Lafont, 1914.

Mansonia: *T. brucei*, Martin, Lebœuf, and Roubaud, 1908; Heckenroth and Blanchard, 1913.

Aëdes (Stegomyia): *T. gambiense*, Roubaud and Lafont, 1914. *T. brucei*, Fülleborn and Meyer, 1907; Roubaud and Lafont, 1914.

Passage of Trypanosomes from Parent to Offspring.—As a general rule, it may be said that the young born of an infected parent are not

themselves infected, and that they are just as susceptible to inoculation as the parent was in the first place. An infected animal often gives birth to still-born young, and in some instances the young born alive have been found infected. The first observation of intra-uterine infection was made by Sivori and Lecler (1902), who noted that a guinea-pig infected with *T. equinum* of mal de Caderas gave birth to an infected young one. Sergent, Ed., Et., and Lheritier (1919) showed that the blood of still-born offspring of camels infected with *T. berberum* was infective to dogs. Sergent, Ed., Et., and Donatien (1920) further reported the finding of trypanosomes in the organs of still-born camels, and noted that if the infection in the parent is in the acute condition, the fœtus becomes infected, while no infection takes place if the parent has clinically recovered, though its blood is still infective to laboratory animals. Bassett-Smith (1919) and Stevenson (1919) showed that trypanosomes occurred in the organs of the fœtuses of rats which were infected with *T. rhodesiense.* Bassett-Smith (1921) also noted that young guinea-pigs born of a parent infected with *T. gambiense* showed trypanosomes in the blood about a month after birth. In this case, the infection may have occurred through the milk. A guinea-pig born of an infected parent and another born of a healthy parent were exchanged. The healthy mother, suckling the infected young one, did not acquire an infection, nor did the healthy young one become infected, though suckled by the infected parent. Nattan-Larrier (1921) showed that *T. cruzi* sometimes passed through the placenta to the fœtus in guinea-pigs.

The question of transmission of trypanosomes from parent to offspring by way of the milk has been studied by Lanfranchi (1915, 1916, 1918, 1918*a*). Infected dogs, cats, guinea-pigs, and rats were used. Milk from infected animals was inoculated into susceptible animals, and infections were produced with *T. brucei*, *T. rhodesiense*, *T. gambiense*, *T. evansi*, and "*T. lanfranchi*" (*T. evansi*). Offspring suckled by infected mothers became infected with *T. brucei*, *T. gambiense*, and "*T. lanfranchi.*" Nattan-Larrier (1913) noted that *T. cruzi* was frequently present in the milk of infected animals and *T. equiperdum* occasionally. Velu and Eyraud (1916) noted that one pup of a litter suckled by a bitch infected with the horse trypanosome of Morocco, *T. moroccanum* (*T. evansi*), acquired the infection. Evans (1880) noted that a pup which was suckled by a bitch infected with *T. evansi* acquired the infection. Kellesberger (1925) has seen a woman and her ten-day-old infant both with trypanosomiasis in the Congo. It would seem probable that this was an instance of intra-uterine infection. The degree of enlargement of the spleen and the number of trypanosomes in the blood of the infant would seem to

exclude the possibility of infection after birth from the milk or as a result of mechanical transmission by insects.

Trypanosomes as Filter Passers.—Various observers have tested the capacity of trypanosomes to pass through porous filters which will not allow the passage of bacteria. In the case of relapsing fever spirochætes it is known that the entire organism, probably on account of its peculiar movements, is able to pass through such filters. In the case of trypanosomes it is the opinion of some observers that this factor cannot account for the passage through the pores of the filter, and that some stage which is smaller than the usual form must exist. Novy and MacNeal (1904*a*) first showed that the passage through a Berkefeld filter of diluted blood containing *T. lewisi* yielded a filtrate which was infective to rats. Experiments with *T. brucei* gave negative results. In these experiments, according to Wolbach, Chapman, and Stevens (1915), the filters had been "thinned down" and were not shown to be impervious to bacteria. Bruce and Bateman (1908) used filters which were proved to prevent the passage of *Micrococcus melitensis*, and found that *T. evansi* from the blood and organ juices of normal animals, and those which had been treated with antimony and the cultural forms from blood-agar medium, could not pass through. Bruce *et al.* (1911*j*) again made similar experiments with the developmental forms of *T. gambiense* in *Glossina palpalis*, but obtained only negative results. Wolbach, Chapman, and Stevens (1915) conducted a very careful series of experiments under varying pressures in which care was taken to prevent the clogging of the pores of the filters. Three trypanosomes were used—*T. gambiense*, *T. brucei*, and *T. lewisi*. The conclusion arrived at is that trypanosomes from cultures and animal tissues are not filterable through bacteria-proof filters. More recently Reich and Beckwith (1922) and Reich (1924) have repeated the experiment. They used the macerated organs of guinea-pigs which had died of *T. brucei* infections. The fluid was filtered after the addition of a loopful of culture of *Bacillus prodigiosus*. The filtrate was immediately inoculated into guinea-pigs, and a control culture was made on glucose agar medium to determine the presence or absence of bacteria. In a series of seventy-two experiments in which the filtrate was free from bacteria, guinea-pigs became infected with *T. brucei* on twenty occasions. A series of seven experiments made with highly infected blood taken from the animals during life gave only negative results. It appears, therefore, that the filterable form is to be found in greatest number in the organs, especially the liver and spleen, of animals which have died of an infection. It does not follow from these experiments that invisible or ultra-microscopic stages of trypanosomes exist. The plasticity of the body compared with that of rigid bacteria would enable an organism to pass through narrow

passages and round corners in which bacteria would become impacted. It has been shown that filters which are impermeable to bacteria on filtration will, nevertheless, allow bacteria to grow through them if sufficient time for multiplication is allowed. It is quite possible that in the experiments of Reich and Beckwith the positive results depend upon the altered trypanosomes in the tissues of dead animals being more plastic and even smaller than those in living animals. Such forms are probably in a degenerate condition, though capable of revival if brought into a favourable environment such as occurs when they are inoculated into a living animal.

Classification of the Pathogenic Trypanosomes.—Many attempts have been made to separate the pathogenic trypanosomes from one another on purely morphological grounds. To a certain extent this can be done, but there are many named species which cannot be recognized from their morphological features alone. Thus, there exist many trypanosomes which are structurally like *T. evansi*, but have been separated by cross-immunity tests and differences in the susceptibility of laboratory animals. It seems to the writer that it has not yet been proved that these tests are of specific value. It would be a remarkable circumstance if *T. evansi*, which in its natural home in India produces surra in horses, had not spread to other countries in view of the extent to which horses have been moved from one part of the world to another during the last two or three hundred years. Many of the trypanosomes of North Africa and South America, for instance, resemble *T. evansi* in their morphology, but have been separated from it as distinct species by the methods mentioned above. It seems more probable that these are merely races of *T. evansi* which have been slightly modified by local conditions after long separation from the parent stock. The same remark applies to the various trypanosomes of the *T. congolense* group. If this view is adopted, then the pathogenic trypanosomes can be grouped in species according to their morphological characters, and the very similar forms which reveal differences in regard to immunity and virulence for laboratory animals only may be considered as races of these. On this basis it is possible to recognize in the tsetse-fly areas of Africa the following forms: *T. brucei* (including *T. rhodesiense*) in man and animals, *T. gambiense* in man and occasionally in domestic animals, *T. congolense* and *T. simiæ* in animals, *T. vivax* in animals (once found in man), *T. capræ* and *T. uniforme* in animals. In tsetse-free areas of Africa, as also in other parts of the world, there is *T. evansi* or its races and *T. equiperdum* in animals.

The pathogenic trypanosomes are often spoken of as being either polymorphic or monomorphic. In this connection the terms apply purely to the appearance of the trypanosomes in the vertebrate host or in inocu-

lated animals. As a matter of fact, when the whole life-cycle is taken into account, all trypanosomes are markedly polymorphic. If it is understood that only the blood stage in the vertebrate is referred to, the term has its application. Thus *T. gambiense*, *T. brucei*, and *T. rhodesiense* are under these circumstances polymorphic. There are two extreme types of trypanosome. One is long and narrow, and has a well-developed flagellum, while the other is shorter and broader, and has no flagellum. A form intermediate between these two types occurs in which there is a short flagellum. The three types are spoken of as "the short stumpy," "the long thin," and "intermediate" trypanosomes. *T. evansi* and its allies—*T. congolense*, *T. simiæ*, *T. vivax*, *T. uniforme*, *T. capræ*, *T. equiperdum*, and *T. equinum*—on the other hand, are monomorphic. In the case of *T. evansi*, *T. equiperdum*, and *T. equinum* all the trypanosomes have flagella, and, unless they are dividing forms, they are of more uniform dimensions than the polymorphic forms, and are very similar to the long narrow forms of *T. brucei*. In *T. vivax*, *T. uniforme*, and *T. capræ* all forms have flagella and a characteristic swollen posterior end of the body. They differ from one another in size. In the case of *T. congolense* and *T. simiæ*, again, the trypanosomes are much smaller than the members of the other groups, and there is no flagellum.

In accordance with these morphological distinctions, there are differences in the developmental cycles in the invertebrate host. Thus the polymorphic forms (*T. gambiense* and *T. brucei*) develop in tsetse flies in the stomach, proboscis, and salivary glands; the *T. congolense* group in tsetse flies in the stomach and proboscis; and the *T. vivax* group in tsetse flies in the proboscis only. *T. evansi* and its allies are not carried by tsetse flies, but are transmitted by tabanids or other biting flies. No developmental cycle has been detected in them. *T. equiperdum*, which is possibly derived from *T. evansi*, is in an anomalous position in that it does not require an invertebrate host.

When the size of any species of trypanosome is referred to, it must be remembered that this is the average size obtained by the measurement of several hundred or a thousand individuals. On the basis of the foregoing facts, the classification of the pathogenic trypanosomes as given on p. 458 (Group B, I. and II.) may be extended as follows:

I. Pathogenic Trypanosomes transmitted by Blood-Sucking Arthropoda.

1. *Pathogenic Trypanosomes transmitted by Species of Glossina.*

(*a*) Development in stomach, proboscis, and salivary glands of the tsetse flies. Polymorphic trypanosomes (short stumpy forms

without flagellum, long thin forms with flagellum, and intermediate forms).

T. gambiense.—Does not show posterior nuclear forms in small laboratory animals.

Size: Length 13 to 32 microns (average 22·5 microns); breadth 1·5 to 3 microns.

Pathogenicity: When established in laboratory animals, very virulent. Much less virulent at first inoculation from man, but monkey most susceptible.

T. brucei (*T. rhodesiense*, *T. pecaudi*).—Shows posterior nuclear forms in small laboratory animals.

Size: Length 12 to 35 microns (average 21 to 23 microns); breadth 1·5 to 3·5 microns.

Pathogenicity: Very virulent for laboratory animals, even at first inoculation from man or other naturally infected hosts.

(*b*) Development in the stomach and proboscis of tsetse flies. Monomorphic trypanosomes with no flagellum.

T. congolense (*T. nanum*, *T. pecorum*).—Size: Length 9 to 18 microns (average 14 microns); breadth 2 to 3 microns.

Pathogenicity: Virulent for all laboratory animals. After passage through goat loses virulence for laboratory animals, and then resembles the natural strain, *T. nanum*, which is not inoculable to laboratory animals.

T. simiæ.—Size: Length 14 to 24 microns (average 18 microns); breadth 1 to 2·75 microns.

Pathogenicity: Not inoculable to small laboratory animals, but highly virulent for monkeys and goats.

(*c*) Development in the proboscis only of tsetse flies.

Monomorphic trypanosomes with flagellum and characteristic swollen posterior end.

T. vivax (*T. cazalboui*).—Size: Length 15·5 to 30·5 microns (average 25 microns); breadth 2 to 3 microns.

Pathogenicity: Not inoculable to laboratory animals as a rule, though rabbits have been infected.

T. uniforme.—Size: Length 12 to 19 microns (average 16 microns); breadth 1·5 to 2·5 microns.

Pathogenicity: Not inoculable to laboratory animals.

T. capræ.—Size: Length 18 to 32 microns (average 25·5 microns); breadth 1·75 to 4·25 microns.

Pathogenicity: Not inoculable to laboratory animals.

2. *Pathogenic Trypanosomes transmitted by Species of Tabanus or Other Blood-Sucking Arthropoda.*

(*a*) Monomorphic trypanosomes with flagellum. Kinetoplast well developed.

T. evansi and many allied forms which are probably races of *T. evansi.*

Size: Length 18 to 34 microns (average 24·9 microns); breadth 1·5 to 2·5 microns.

Pathogenicity: Virulent for all laboratory animals.

(*b*) Monomorphic trypanosomes with flagellum. Kinetoplast rudimentary.

T. equinum.—Size: Same as *T. evansi.*

Pathogenicity: Virulent for all laboratory animals.

II. Pathogenic Trypanosomes Secondarily adapted to Direct Passage from Vertebrate to Vertebrate.

(*a*) Monomorphic trypanosomes with flagellum.

T. equiperdum.—Size: Length 16 to 35 microns (average 24 microns); breadth 1·5 to 2·5 microns.

Pathogenicity: When established, virulent for all laboratory animals, but very difficult to inoculate from horse in the first place. Dog most susceptible.

According to this scheme, the above trypanosomes can be separated from one another on morphological grounds, with the possible exception of *T. equiperdum*, which structurally is closely related to *T. evansi.*

1. PATHOGENIC TRYPANOSOMES TRANSMITTED BY SPECIES OF GLOSSINA.

(*a*) Trypanosomes which Develop in the Stomach, Proboscis, and Salivary Glands of Tsetse Flies.—Polymorphic Trypanosomes

Trypanosoma gambiense Dutton, 1902.—Synonyms: *T. ugandense* Castellani, 1903; *T. castellanii* Kruse, 1903; *T. hominis* Manson, 1903; *T. fordii* Maxwell-Adams, 1903; *T. gambiæ* Maxwell-Adams, 1903; *Trypanozoon gambiense* (Lühe, 1906); *Trypanosoma rovumense* Beck and Weck, 1913; *T. tullochii* Minchin, 1907; *T. nigeriense* Macfie, 1913; *Castellanella gambiense* (Chalmers, 1918); *C. castellanii* (Chalmers, 1918).

T. gambiense was first seen by Ford in the blood of a man in the Gambia, and was recognized as a trypanosome and named by Dutton (1902). In the following year, Castellani described as *T. ugandense* a trypanosome he found in the cerebro-spinal fluid of a case of sleeping sickness in Uganda, an observation which was soon confirmed by Bruce and Nabarro (1903). Following these discoveries, it was definitely established that the disease known as sleeping sickness was merely the final stage of human trypano-

somiasis, and that the trypanosomes of Dutton and Castellani were identical. Hence, *T. gambiense* stands as the correct name, while the other names become synonyms.

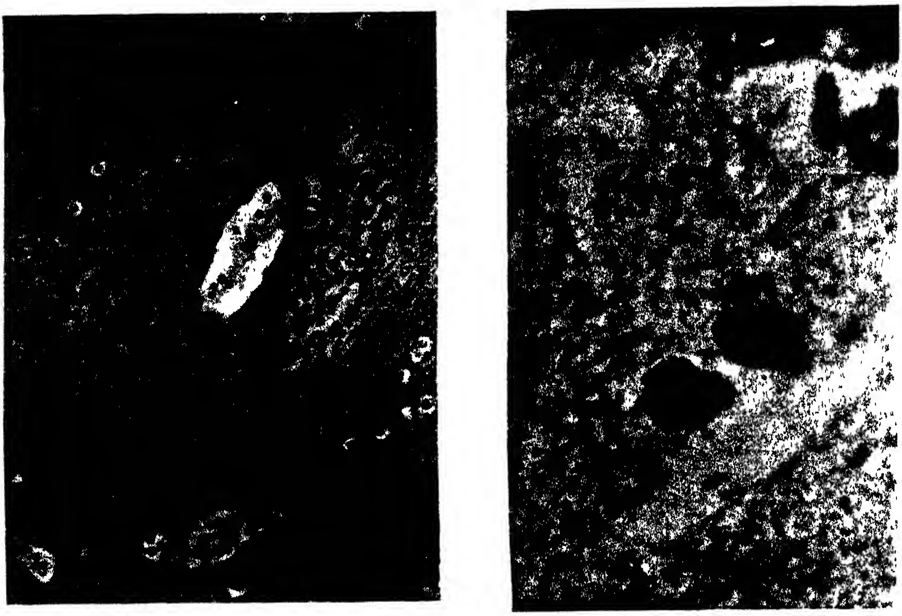
Distribution.—*T. gambiense* occurs only in Africa. On the West Coast it is limited to a district between 15° N. and 15° S. of the equator. Further east, it is restricted to the area between 10° N. and 10° S., its eastern limits being Lakes Victoria and Tanganyika. In these regions it occurs in greatest intensity along the rivers and shores of the great lakes, and, wherever it is found, there the tsetse fly, *Glossina palpalis*, also occurs. This association of the fly with the infection in man led to it being suspected as the carrier of the disease, but the actual part played by the fly was never properly explained till Kleine made his observations on the behaviour of trypanosomes in tsetse flies, the first account of which was published in 1909.

Symptomatology.—In the blood of man the trypanosome never occurs in great numbers, and long search may be required to discover it. Sometimes it can be more readily found by examination of fluid obtained by puncture of an enlarged lymphatic gland, and later in the disease in the cerebro-spinal fluid. At other times its presence has only been demonstrated by inoculation of fairly large quantities of blood (20 c.c. or more) into some susceptible animal like the monkey. As a rule, however, careful search of one or more ordinary blood-films will reveal its presence.

This may have to be repeated on several occasions, for, as is usual in these infections, the number of trypanosomes in the blood is subject to definite fluctuations. Age and sex do not appear to influence infection to any extent unless, owing to habits of occupation, any particular age or sex is more liable to exposure than another.

It has been noted in certain districts of Africa that natives may be found to harbour the trypanosome, though apparently perfectly healthy. On this account it may be difficult to give a definite incubation period, which is said to vary between two or three weeks and seven years. Generally, the first symptom noted is irregular fever, which is uncontrolled by quinine. This is followed by enlargement of the lymphatic glands and spleen, anæmia, and wasting. A cutaneous eruption in the form of circular red patches may occur. This stage is due to the invasion of the blood and lymphatic system by the trypanosomes. A second stage is due to the extension of the infection to the central nervous system, during which various nervous symptoms become manifest, leading finally to that lethargic condition which has given rise to the name sleeping sickness. Recovery frequently takes place as a result of treatment in the first stage of the disease, but more rarely when the second stage is reached (see p. 459).

Pathology.—The lesions produced in man by *T. gambiense* consist of a hyperplasia of the lymphatic tissue of the body. There is enlargement of the lymphatic glands and spleen. In the later stages the meninges are affected, while there is an increase in the cerebro-spinal fluid. Most marked are the changes about the arteries of the brain and cord, leading to a thickening of the arterial coat, together with invasion of the area around the vessel by round cells, which give rise to the characteristic round-celled infiltration (Fig. 221, A). Mott (1899, 1906) pointed out that



A B

FIG. 221.—SECTION OF BRAIN OF FATAL CASES OF SLEEPING SICKNESS. (AFTER STEVENSON, 1922 AND 1923; FROM *Trans. Roy. Soc. Trop. Med. and Hyg.*, vol. xi.)

A. Perivascular infiltration by round cells (× 200).
B. Frontal lobe, showing two trypanosomes in grey matter (× *ca.* 1,000).

the lesions in sleeping sickness resembled those of general paralysis. As a rule, in animals which do not live long after inoculation, these lesions are not apparent, but in monkeys with long-standing infections, and even in smaller animals if they survive for several months, the same round-celled infiltration about the cerebral vessels can sometimes be demonstrated. In sections of the tissues of man, trypanosomes are with difficulty found, but their distribution in guinea-pigs infected with a strain of *T. gambiense* from Nigeria has been studied by Stevenson (1917, 1918). Though present

in the blood-stream in very small numbers, they may be found in the lymph channels in any part of the body in greater numbers. They do not appear to be intracellular, but can be found between the cells of the brain, heart, stomach, kidney, and, in fact, any organ of the body where small patches, probably of an œdematous nature, occur in which the trypanosomes are considerably more numerous than elsewhere (Fig. 221, B). Similar results were previously obtained by Wolbach and Binger (1912), who studied the distribution of *T. gambiense* in rats, guinea-pigs, and monkeys, and by Yorke (1911) in the case of *T. brucei* (*T. rhodesiense*) in goats.

Stevenson (1922) examined the brain of a fatal case of sleeping sickness in which trypanosomes had not been seen in the blood for many months, though careful search had been made. There was a high degree of round-celled infiltration of the vessels of the brain, and trypanosomes could be demonstrated in the intercellular spaces of the brain substance (Fig. 221). These observations seem to indicate that the site of election of the trypanosomes is not the actual blood-stream itself, but rather the lymphatic channels. In this connection it is of interest to note that abstraction of fluid from lymphatic glands as a means of discovering trypanosomes more readily than in the blood was first advocated by Greig and Gray (1904) as a means of diagnosing the disease in man. Clapier (1921) observed trypanosomes in large numbers in the fluid abstracted from hydroceles and Newham (1919) in peritoneal exudate.

In the case of heavy infections in experimental animals, smears from the spleen, bone marrow, or other organs may show trypanosomes in an intracellular position. This is a result of phagocytosis, and the trypanosomes are quickly destroyed. Phagocytosis of this kind occurs in the case of other trypanosomes, and the process as it occurs *in vitro* in the case of *T. lewisi* was described by Laveran and Mesnil (1904). In the process of digestion the trypanosomes assume various forms which must not be mistaken for developmental stages. The trypanosomes which occur in the cerebro-spinal fluid of human beings in sleeping sickness often have a curiously abnormal shape.

There appear to be no data to indicate how early in the disease trypanosomes enter the cerebro-spinal fluid. Broden and Rodhain (1908) state that the degree of involvement of the central nervous system can be gauged by the cell content of the cerebro-spinal fluid. A normal fluid contains not more than three lymphocytes per cubic millimetre. In the earliest stages of involvement there is an increase in the lymphocytes. Later there appear medium-sized mononuclear cells, and still later large vacuolated mononuclear cells. In a series of cases which were clinically in an advanced stage Pearce (1921) found the number of cells in 1 c.mm.

of cerebro-spinal fluid to vary from 15 to 467. The blood leucocytes in these cases varied from 4,500 to 12,000 per c.mm.

Keratitis is common in animals infected with pathogenic trypanosomes, and it occurs less commonly in man. In the case of animals, Morax (1906, 1907) and Yorke (1911) showed that the condition was due to the presence of large numbers of trypanosomes in the lymph spaces, which were often swollen and œdematous.

Morphology.—*T. gambiense*, after its inoculation into man by *Glossina palpalis*, presumably invades the blood and lymphatic channels, and there multiplies by repeated longitudinal division. On account of its scarcity in the blood of man, its morphology has been studied chiefly in the blood of susceptible animals. In the blood of such an animal as the rat or guinea-pig the trypanosome varies in length between 15 and 30 microns, and, as pointed out by Minchin (1908), occurs in three types, for which reason it is regarded as a polymorphic trypanosome (Plate V., A, p. 456). There is a short and broad form which has no flagellum, a long thin form with a flagellum, and an intermediate form (Fig. 222, A-C). These three types are not sharply defined, as they merge into one another by inappreciable gradations. Robertson (1912*b*) has shown that the short forms are the result of division of the long ones, and that they grow into long forms which divide. The majority of trypanosomes in any infection come within the dimensions given above, but longer forms are sometimes seen nearly 40 microns in length. These are generally dividing forms. On the other hand, trypanosomes less than 15 microns in length are seen, especially in inoculated rats and guinea-pigs. The occurrence of peculiar short stumpy forms in the blood of a guinea-pig inoculated with the South Nigerian human trypanosome combined with its low virulence for human beings, especially in children, led Macfie (1913*a*) to suggest its separation as a distinct species, *T. nigeriense* (Fig. 222, D). As these very short forms occur in rats inoculated from human beings with undoubted *T. gambiense*, and as the virulence of this trypanosome for man varies considerably in other parts of Africa, it is highly probable that Macfie's trypanosome is merely a strain of *T. gambiense* of exceptionally low virulence.

In *T. gambiense* the nucleus occupies a central position and the kinetoplast a point a short distance from the posterior end of the body. As regards the undulating membrane, it is of moderate width and not markedly convoluted, being more so than in *T. lewisi* and less so than in some of the other pathogenic trypanosomes. Granules of volutin may or may not be present in the cytoplasm. Multiplication is by longitudinal division in the usual manner, and calls for no special remark. The supposed sexual process associated with the production of "latent bodies" described by

Moore and Breinl (1907) has already been considered (p. 332). It may be noted that the occurrence of short broad forms and long thin trypanosomes has been supposed to indicate a sexual dimorphism, of which at present there is no evidence. Robertson (1912*b*) considers that the short broad

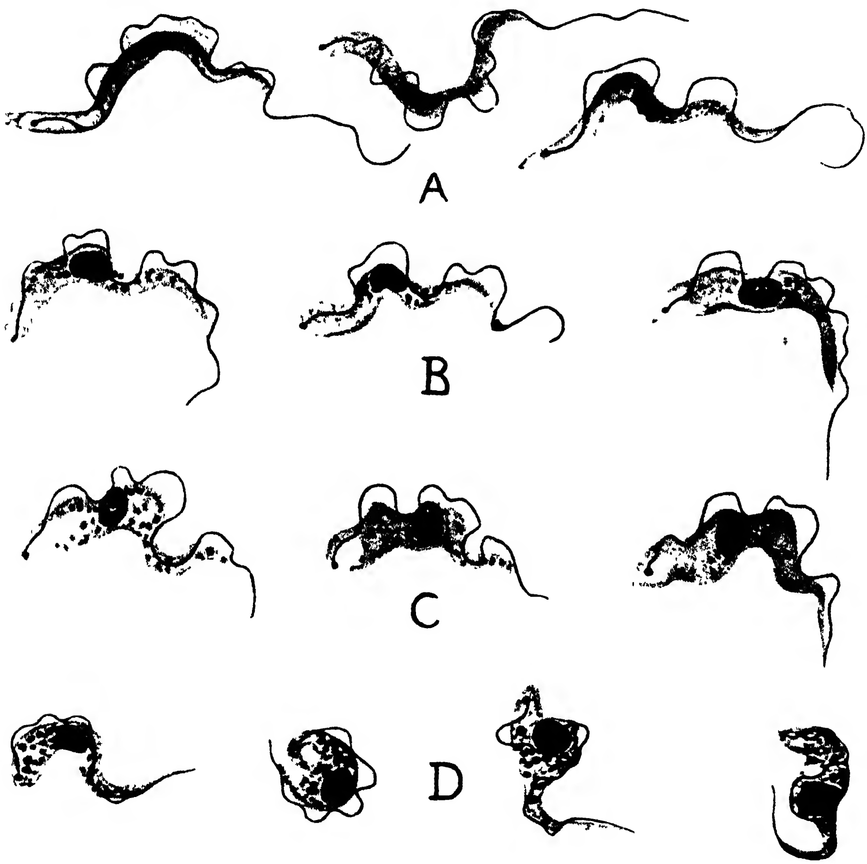


FIG. 222.—*Trypanosoma gambiense* (×2,000). (A, B, AND C, AFTER BRUCE, 1912; D, ORIGINAL.)

A. Long thin form with flagellum. B. Intermediate form with short flagellum.
C. Broad stumpy form without flagellum.
D. Small forms from a rat inoculated with the Nigerian strain.

trypanosome 13 to 20 microns in length is the adult blood form. When proceeding to divide, growth takes place till the long forms are produced. These are the dividing individuals, which give rise to the short broad forms again. Robertson (1912*b*) believes that the short broad forms alone are capable of development in the tsetse fly.

As already remarked, trypanosomes occur in the cerebro-spinal fluid. Here they may be found in the later stages of the disease, and are peculiar in that they exhibit a marked want of uniformity in size and shape. Curious rounded, stumpy, or pear-shaped forms are often encountered. These are to be regarded as abnormal or involution forms, and are of no special significance in the life-history of the trypanosome.

Susceptibility of Animals.—It is possible to inoculate *T. gambiense* into all the ordinary laboratory animals. Monkeys are readily infected, but baboons (*Cynocephalus*) are refractory. Those of the genera *Macacus*, *Cynomolgus*, and *Cercopithecus* (especially *C. ruber*) are very susceptible. The smaller animals such as rats, mice, guinea-pigs, and rabbits are not so readily infected as monkeys when the inoculations are made directly from man. After a strain has passed through a monkey, it becomes more virulent for the smaller animals. The dog and the cat are susceptible, as also goats, sheep, horses, and cattle. Fowls are inoculated with difficulty. The course of infection in these animals varies considerably. The virulence of any particular strain increases with passage from animal to animal till it causes death in two to three weeks in rats and guinea-pigs, and even in monkeys. The first passage from man may, in small animals, give rise to an infection lasting several months, or even a year or more, during which time trypanosomes are with difficulty discovered in the blood. In the larger domestic animals the infection is of a mild and chronic type, trypanosomes often being demonstrable only by inoculation of their blood into more susceptible animals.

Animal inoculations are of service as an aid to diagnosis. One or two cubic centimetres of blood from a man may be inoculated intraperitoneally into a rat or guinea-pig, or larger quantities into a monkey. It must be remembered that a failure to produce infection does not prove that trypanosomes are absent. In the writer's experience, inoculation of fairly large quantities of blood known to contain trypanosomes into rats has not infrequently failed to give rise to any recognizable infection.

Culture.—In the usual blood media, as, for instance, N.N.N. medium, which answers well for many flagellates of this group, *T. gambiense* may survive for a fortnight or more, and show changes of structure to the crithidia type, but it does not multiply to any extent, so that the maintenance of a culture by sub-inoculation of fresh tubes does not succeed, though the flagellates transferred may linger for a week or more before finally disappearing. Media which contain a comparatively large proportion of human blood give better results than others, but the satisfactory maintenance of *T. gambiense* outside the body has not yet been accomplished.

Transmission.—*T. gambiense* is conveyed from man to man by the tsetse fly, *Glossina palpalis*, which has a distribution in Africa corresponding fairly closely with that of the trypanosome (Fig. 223). After Bruce (1895) had demonstrated the carriage of *T. brucei* by *G. morsitans*, many prophetic utterances as to the probable transmission of *T. gambiense* by tsetse flies were made. The first of these was that of Brault (1898), who,

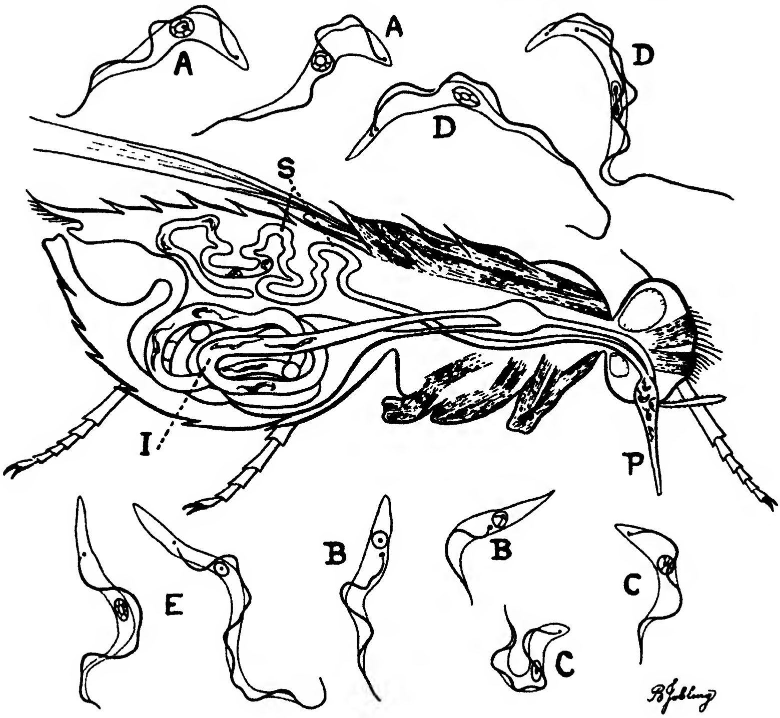


FIG. 223.—DIAGRAM OF *Trypanosoma gambiense* IN THE BLOOD OF MAN AND THE TSETSE FLY (*Glossina palpalis*). (AFTER WENYON, 1922.)

A. Ordinary forms in the blood of man. D. Dividing forms in the blood of man.
P. Trypanosomes passing through proboscis of fly.
S. Trypanosomes in stomach of fly. I. Trypanosomes in salivary gland of fly.
E. Long trypanosomes which make their way from the stomach to the salivary glands via the proboscis.
B. Crithidia forms developed from the long trypanosome forms in the salivary glands.
C. Metacyclic trypanosomes developed from the crithidia forms in the salivary glands. These forms produce infection when injected with the salivary secretion of the fly.

before the actual discovery of *T. gambiense*, predicted that sleeping sickness would probably be found to be a disease caused by trypanosomes and transmitted by tsetse flies. Bruce, Nabarro, and Greig (1903), working in Uganda with wild *G. palpalis* caught off animals, showed that batches of flies fed on sleeping sickness cases were able to transmit infection to monkeys up to forty-eight hours after feeding. Combined with the fact

that trypanosomes (*T. brucei*) had been seen by Bruce (1897) in the proboscis up to forty-six hours after feeding, the conclusion was arrived at that the transmission was a mechanical one. The experiments carried out by Bruce, Nabarro, and Greig (1903), however, indicated that a cyclic development in the fly was not excluded, for freshly caught flies were shown to infect monkeys. It was shown by Minchin (1908) and others that in mechanical transmission it was not possible for a fly to infect more than one animal, the proboscis being apparently purged of trypanosomes at the first feed. Minchin, Gray, and Tulloch (1906), and Minchin (1908), working with single flies (*G. palpalis*), transmitted *T. gambiense* nine times out of ten by the method of interrupted feeding, by which the flies were allowed to commence their feed on an infected animal and to complete it on a healthy one. Bruce *et al.* (1910*f*), by using laboratory bred flies, proved that mechanical transmission of *T. gambiense* by *G. palpalis* could take place within two hours of feeding. It was recognized that in the earlier experiments noted above, when wild flies were used, what had been regarded as mechanical transmission after forty-eight hours was probably due to the flies having already been infected in nature.

Much confusion regarding the behaviour of trypanosomes in the flies was at first caused by *Herpetomonas grayi*, which was not distinguished from *T. gambiense* (Figs. 173 and 220). Minchin, Gray, and Tulloch (1906) first showed that this flagellate was distinct from *T. gambiense*, a fact which was recognized later by Novy (1906), who examined films sent him by Gray. After *H. grayi* had been recognized, the behaviour of *T. gambiense* in *G. palpalis* was studied by Minchin, Gray, and Tulloch (1906), who found that the ingested trypanosomes disappeared entirely from the gut of the fly in four days. This led Minchin (1908) to express the view that *T. gambiense* in Uganda was transmitted by *G. palpalis* in a purely mechanical manner; though influenced by the work of Koch (1905) and Stuhlmann (1907), chiefly on *T. brucei*, he still held that, given the proper conditions and the proper fly, a true cyclic development would be found to take place. Koch (1905) noted that a fluid free from red blood-corpuscles and containing large numbers of trypanosomes could be expressed from the proboscis of wild tsetse flies, *G. fusca* (? *G. brevipalpis*), *G. morsitans*, and *G. palpalis*. From what is now known, these trypanosomes, which were of the vertebrate blood type, were undoubtedly the metacyclic infective forms. It was probably these forms which Gray and Tulloch (1905) found in the salivary glands of a fly. Stuhlmann (1907) confirmed Koch's observations, and also noted long narrow forms in the proventriculus. He found that from 3 to 14 per cent. of wild tsetse flies, *G. fusca* (? *G. brevipalpis*), had trypanosomes in the proboscis. Roubaud (1908) also obtained trypanosomes from the proboscis of wild flies. Bruce

(1903) had shown in Zululand that the trypanosomes which develop in the stomach of the tsetse flies are not infective to animals, an observation which was confirmed by Koch (1905), Gray and Tulloch (1905), Minchin, Gray, and Tulloch (1906), and Bouet (1907). Minchin (1908) remarks that Manson made the suggestion that this lack of infectivity was due to the trypanosomes being in a developmental stage, and was in favour of a developmental cycle in the fly. Other observers, notably Cazalbou (1906), Dutton, Todd, and Hannington (1907), Bouet (1907), Roubaud (1907), Ross, P. H. (1908), and others, made contributions to the subject without, however, solving the problem. They all effected transmission of trypanosomes by means of tsetse flies fed first on infected animals and shortly after on healthy ones. As wild flies were used, it is highly probable that some of the flies were already infected when caught. Ross, P. H. (1907), succeeded in infecting a monkey with what he regarded as *T. gambiense* by means of wild *G. pallidipes*, and in the following year (1908) a monkey with the same trypanosome by *G. fusca* feeding alternately on an infected and the healthy animal. There is, however, considerable doubt as to the species of trypanosome used in these experiments. Kleine (1909*a*), working in German East Africa with *T. brucei*, discovered that laboratory bred flies do not become infective till after the expiry of about twenty days from their infecting feed. This important observation proved conclusively that a definite cycle of development took place in the fly, and explained the failure of the earlier observers, who did not extend their experiments over a sufficiently long period after feeding the flies on infected animals. Kleine's experiments were conducted with *T. brucei* and *G. palpalis*, but his results were quickly confirmed by Bruce *et al.* (1909, 1910*a*, 1911*d*), working in Uganda with *T. gambiense* and *G. palpalis*. The important fact of the necessary incubation period in tsetse flies having been established, it was soon demonstrated by Bruce *et al.* (1911*d*) that *T. gambiense* went through a cycle of development terminating in infection of the salivary glands, where infective metacyclic trypanosomes appeared (Fig. 223). Bruce *et al.* (1911c) showed that after ingestion by the fly, *T. gambiense* in the stomach remained infective to inoculated animals for two days, after which the infectivity was lost. The forms which appear in the salivary glands eventually become capable of infecting animals, the period of non-infectivity of the fly forms corresponding with that during which the fly is unable to transmit the infection by its bite.

Though *G. palpalis* is the host of *T. gambiense*, not every fly which feeds on an infected animal becomes infective. The percentage of flies in which the developmental cycle completes itself varies, but it is well under 10 per cent. As regards the transmission of *T. gambiense* by other species of tsetse flies there is some experimental evidence. Rodhain,

Pons, Vandenbranden, and Bequært (1912*b*) succeeded in transmitting *T. gambiense* by means of laboratory bred *G. morsitans* fed on infected monkeys. Kleine and Fischer (1912) also succeeded in a similar experiment, as did Bruce *et al.* (1915). Taute (1911) fed *G. morsitans* on a monkey which had been infected from a man. These flies later infected healthy monkeys. Lloyd and Johnson (1924), working in North Nigeria, have transmitted *T. gambiense* by means of *G. tachinoides*, in which a complete development of the trypanosomes occurred. It appears that in certain areas this fly is responsible for the spread of sleeping sickness. These instances can only be regarded as exceptions to the general rule that *T. gambiense* is transmitted in nature by *G. palpalis*.

T. gambiense may also be transmitted in a mechanical manner by mosquitoes and other biting flies. Thus, Heckenroth and Blanchard (1913) showed that mosquitoes (*Mansonia uniformis*) could infect guinea-pigs within twenty-four hours of feeding on an infected animal, while Minchin, Gray, and Tulloch (1906) were successful with *Stomoxys* which had partially fed on an infected animal and had completed its feed on a healthy one. In the latter case, the trypanosome transmitted was possibly *T. brucei*, and not *T. gambiense*. Duke (1919, 1923) has come to the conclusion that mechanical transmission of a virulent strain of *T. gambiense* from man to man was largely responsible for the spread of sleeping sickness through Uganda from 1900 to 1910. He believes that when human trypanosomiasis occurs in epidemic form, mechanical transmission is responsible for the rapid spread of the disease, while transmission associated with the cycle of development in the fly maintains the disease in endemic form.

Cycle in the Tsetse Fly.—The main outline of the development of *T. gambiense* in *G. palpalis* (Fig. 223) was described by Bruce *et al.* (1911*d*). It was studied in greater detail by Robertson (1913), whose account is followed here.

When a fly hatched from the pupa ingests blood and trypanosomes from an infected animal, one of several alternatives may occur.

1. The trypanosomes may be destroyed and disappear during the fifty to seventy-two hours during which the blood is being digested.

1. Trypanosome of the blood-stream. 2. Division of blood form.
3. Trypanosome in mid-gut thirty-six to forty-eight hours after feeding.
4-6. Trypanosome in hind-gut third or fourth day of cycle.
7. Trypanosome in mid-gut on fifth day.
8. Large multiple form (delayed division) sixth day.
9-11. Trypanosomes in gut twelfth to twentieth day.
12-13. Slender proventricular types—final form of gut development.
14-15. Form newly arrived in salivary glands.
16-20. Typical salivary gland—crithidia forms.
21-23. Final trypanosome types in salivary glands (metacyclic trypanosomes).

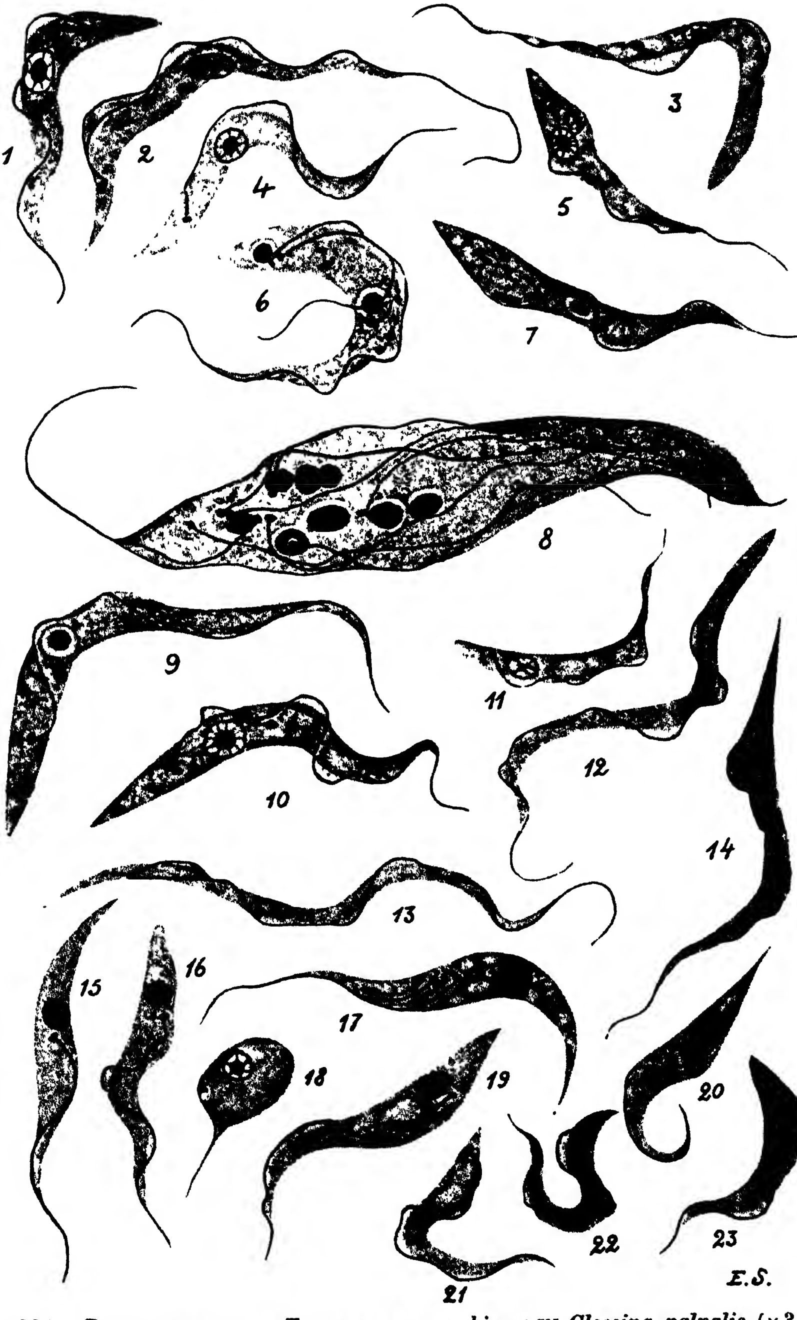


FIG. 224.—DEVELOPMENT OF *Trypanosoma gambiense* IN *Glossina palpalis* (× 3,000) (AFTER ROBERTSON, 1913.)

[*For description see opposite page.*

2. Trypanosomes may not entirely disappear with digestion of the first feed of blood, but do so at the second.

3. They may survive and multiply in the gut, although a second feed of blood has been superadded.

4. They may survive and multiply in the crop for as long as twelve days, provided the crop has never been entirely emptied of blood. In such cases the gut may be entirely free from trypanosomes. Those in the crop are unable to survive a complete emptying of the organ, and no permanent infection of the fly results if this takes place.

5. The trypanosomes may persist in greater or less numbers both in the gut and in the crop of the same fly.

6. The whole of the partially digested blood which survives from the first feed may be displaced by the fresh blood of the second feed without the trypanosomes which are present in the stomach disappearing. The crop in these cases may be either empty or filled with new blood.

Of these various conditions, any one of which may result from a feed of infected blood, the last appears to be the one which leads to a permanent infection of the fly. It is thus evident that only a small percentage of the flies actually fed will acquire an infection. In the flies in which infection will occur, active multiplication of the trypanosomes in the mid- and hind-gut commences soon after the infecting feed, and continues progressively. There is no intracellular stage of the trypanosomes, no stage in which they are attached to the gut wall, and in no case do they disappear from the gut to reappear at a later period. Reproducing trypanosomes are thus constantly present in the lumen of the gut.

Thirty-six to forty-eight hours after ingestion many degenerating trypanosomes are present, together with dividing healthy forms, which appear to be all of the short broad type which were present in the blood of the vertebrate. They differ little at this stage from the blood type (Fig. 224, 1-2), though the undulating membrane may be a little straighter and the kinetoplast slightly displaced towards the nucleus (Fig. 224, 3). The division results in the formation from the parents of daughter individuals, which exceptionally may have, for a short time only, the crithidia arrangement of nucleus and kinetoplast. The trypanosome arrangement, however, is quickly regained. These crithidia forms only occur at the early divisions, and they are the only indication of a crithidia phase in the intestinal development. By the tenth day a large number of trypanosomes remarkable for the variety of their shape and size has been produced, but the maximum length attained by any one does not exceed 35 microns (Fig. 224, 4-11). At this stage there may appear a small number of characteristic slender individuals. From the tenth to the fifteenth day these slender forms are developed gradually from the broader

forms in increasing numbers. They are to be regarded as the regular proventriculus type, and differ from the broad forms which they are replacing in having a more finely granular cytoplasm, a nuclear karyosome reduced in size, and a nuclear membrane which stains more deeply (Fig. 224, 12-13). Division of the slender forms may still take place. During this multiplication period there are produced an enormous number of trypanosomes, which invade the anterior part of the mid-gut, and finally the anterior gut and proventriculus. The anterior part of the anterior gut and the proventriculus contain the long slender trypanosomes which invade this portion of the intestine between the tenth and twentieth day. Prolonged fasting causes the infection to pass back again till it becomes limited to the middle and hind part of the mid-gut. A fresh feed again brings the infection forwards to the proventriculus. If, however, new blood is taken in while the proventriculus infection exists, the trypanosomes maintain their position. The long slender trypanosomes are the forms which were seen by Stuhlmann (1907) in the proventriculus.

The further development is brought about by the slender proventriculus forms passing into the labial cavity and hypopharynx, and thence into the narrow tubular portion of the salivary gland, which consists of a narrow tube, a broader cellular part, and finally the still broader terminal glandular portion (Fig. 224, 14-16). The trypanosomes attach themselves to cells of the cellular part or commencement of the glandular part. They gradually transform into broad crithidia forms with rounded posterior ends (Fig. 224, 16-20). They multiply, and gradually the cavity of the gland becomes filled with flagellates which vary in shape and size between tadpole-shaped crithidia forms and trypanosome forms resembling very closely the vertebrate blood type (Fig. 224, 20-23). Division of all these forms takes place, the crithidia forms being mostly attached to the surface of the glandular cells. Fresh slender trypanosomes are constantly travelling up the duct from the hypopharynx, so that there is a continuous production of fresh crithidia forms, which in their turn produce the trypanosomes of the blood type. The flies seem to become infective from two to five days after the slender forms invade the gland. The whole development occupies about twenty days. The cytology of the gland forms calls for no special remarks except that their nuclei appear to be richer in chromatin than those of the slender invading forms.

It will thus be seen that there is an intestinal multiplication phase of the trypanosome forms, leading to the formation of slender trypanosome individuals which invade the proventriculus and hypopharynx. These pass to the salivary glands by way of the duct, and become flagellates of the crithidia type, which in turn gives rise to metacyclic trypanosomes, closely resembling the blood type which commenced the developmental

cycle in the gut (Figs. 217 and 223). As the glands are not infective when injected into animals till the final trypanosome stages appear, the latter are the actual infective metacyclic forms. During the whole of this development no sexual process was observed. In spite of failure to observe it, Robertson considers that there is a good deal of circumstantial evidence that a conjugation or some equivalent process takes place. The passage through the fly seems to have some biological significance in playing an "essential rôle in maintaining the integrity of the species. quite apart from its being a convenient method of transmission." If a sexual process occurs, it will probably be at that stage "which is absolutely essential to the production of a trypanosome viable in the blood of the vertebrate—namely, the crithidial phase in the salivary gland."

Reservoir Hosts.—Owing to the increase of sleeping sickness along the shores and on the islands of Lake Victoria Nyanza, the prophylactic measure of removing the native population was adopted. Though this was carried out, five years later Duke (1915) found that two fly-boys who had been bitten by *G. palpalis* on the lake shore or islands contracted sleeping sickness, an inadvertent experiment which proved that reservoirs of *T. gambiense* still existed in the locality. Bruce *et al.* (1911*e*) failed to find *T. gambiense* in animals examined on the lake shore. Duke (1912*a*, 1912*c*), however, was able to demonstrate that the sitatunga, *Tragelaphus spekei*, harboured a trypanosome which he regarded as *T. gambiense.* It was concluded that this antelope was acting as a reservoir for the virus in 1915. But whether the flies acquired their infection only from this source must be doubtful, for more recent observation seems to indicate that the islands had not been kept so free from human beings as was at first supposed. It was proved by Gray and Tulloch (1907) that the dogs of Uganda in endemic areas of sleeping sickness might harbour what was apparently *T. gambiense,* an observation which was also made, according to Koch, Beck, and Kleine (1909), by Van Someren. Bruce and his co-workers (1910*c*) showed that cattle might act as a reservoir for *T. gambiense.* Healthy animals could be experimentally infected by *G. palpalis,* and in the fly area they found a naturally infected cow. In a similar manner, antelope (water buck, bush buck, reed buck) could be infected with *T. gambiense,* while bred flies fed on these animals became infected. No antelope, however, were found naturally infected, though trypanosomes were found in a monkey (*Cercopithecus pygerythrus centralis*) from the lake shore. Fraser and Duke (1912) showed that antelope may remain in perfect health for over a year after experimental infection with *T. gambiense,* and that *G. palpalis* could be infected from them 315 days after inoculation. Blood from an antelope 327 days after inoculation was still capable of producing infection in rats. Kleine and Taute (1911) succeeded

in infecting sheep and goats in the same manner. Prolonged search amongst antelope by Bruce, Kleine and Fischer, Duke and Fraser, and other workers for a reservoir host for *T. gambiense* did not meet with any success, except in the case of the sitatunga noted above. Koch, Beck, and Kleine (1909), and Bruce *et al.* (1911*e*) reported having found monkeys naturally infected with trypanosomes resembling *T. gambiense.* Trypanosomes which were possibly *T. gambiense* were seen by Kleine and Eckard (1913) in a cow, a sheep, and a goat in Tanganyika, by Duke (1913*a*) in a buffalo and a hyena in Western Uganda, by Yorke and Blacklock (1915) in a cow in Sierra Leone, and by Simpson (1918) in a reed buck in the Gold Coast. In none of these cases can it be taken for granted that the trypanosome was certainly *T. gambiense.* Unless studied in small laboratory animals, it is impossible to distinguish *T. gambiense* from *T. brucei*, and in most of these instances of supposed infection with *T. gambiense* this was not done. Even Duke's observation (1912*a*, 1912*c*) on the sitatungas, which has been generally accepted, is open to doubt, for, reinvestigating these animals (1921), he found that the trypanosomes with which they were naturally infected produced posterior nuclear forms when injected into guinea-pigs, and were more virulent than those previously isolated. He concludes that *T. gambiense*, by long residence in the sitatunga, has reverted to the *T. brucei* type, but at the same time admits that during the earlier investigations the significance of posterior nuclear forms was not realized, so that they were not specially looked for, and may have been neglected. It would seem, therefore, that search for a reservoir host for *T. gambiense* has shown that occasionally domestic animals living in association with human beings amongst whom the disease occurs may acquire an infection, but there is little or no evidence to incriminate the wild game as reservoirs of this trypanosome. There does not appear to be such a close connection between *T. gambiense* and the wild fauna of Africa as in the case of *T. brucei.* Probably, therefore, man himself, and sometimes the domestic animals near him, are most usually the sources from which *G. palpalis* derives its infection. *T. gambiense*, which undoubtedly originated from a trypanosome of animals (probably *T. brucei*) in the first place, has now become adapted to man to such an extent that there is little tendency for it to infect the game. In this respect it stands in marked contrast to the human strain of *T. brucei* (*T. rhodesiense*).

Trypanosoma brucei Plimmer and Bradford, 1899.—Synonyms: *T. suis* Ochmann 1905; *Trypanozoon brucei* (Lühe, 1906); *T. suis* (Lühe, 1906); *Trypanosoma pecaudi* Laveran, 1907; *T. togolense* Mesnil and Brimont, 1909; *T. elephantis* Bruce *et al.*, 1909; *T. rhodesiense* Stephens and Fantham, 1910; *T. anceps* Bruce *et al.*, 1914; *T. ugandæ* Stephens and Blacklock, 1913; *Castellanella brucei* (Chalmers, 1918); *C. rhodesiense* (Chalmers, 1918); *T. multiforme* Kinghorn and Yorke, 1913 (?); *T. equi* Blacklock and Yorke, 1914 (?); *T. dukei* Knuth and du Toit, 1921.

This trypanosome was discovered by Bruce in 1895, and proved to be one of the causes of nagana, a disease which had long been known to attack domestic animals in Zululand. Accounts of his observations were published in 1897 and 1903. The trypanosome was named *Trypanosoma brucii* (*T. brucei*) by Plimmer and Bradford (1899), from the forms which occurred in an infected dog which had been sent to England by Bruce.

Stephens and Blacklock (1913) noted that the strain was monomorphic, and resembled *T. evansi* rather than the polymorphic form here described as *T. brucei*. Plimmer and Bradford (1899) described their trypanosomes as monomorphic, hence Stephens and Blacklock think that the polymorphic Uganda trypanosome, which is now generally called *T. brucei*, cannot be the same as the original monomorphic Zululand trypanosome, the true *T. brucei*. They therefore suggest the name *T. ugandæ* for the polymorphic form. Bruce, on the other hand, regards his original Zululand strain as the same as the polymorphic form now generally known as *T. brucei*, and ascribes the discrepancy to a change in type which has probably taken place owing to long maintenance in laboratory animals. That some profound change had taken place receives support from Roubaud's observations (1913) that the Pasteur Institute strain was no longer capable of infecting *Glossina morsitans*. The writer has noted on several occasions that trypanosomes inoculated from the blood of man into laboratory animals may show posterior nuclear forms at first, and that these disappear entirely in subsequent passages, the trypanosomes tending to become more and more monomorphic. The figure given by Bruce (1897) of the trypanosome in the blood of the dog shows definitely a polymorphic form, while one of the trypanosomes appears to have the nucleus in a posterior position.

There can be no doubt that one of the trypanosomes causing nagana is really a polymorphic trypanosome, whatever Plimmer and Bradford said about the strain they examined in 1899. It is quite possible that they overlooked or neglected to describe the forms without flagella, a point which can only be determined by a re-examination of their original films. It is worthy of note that, though *T. brucei* had been studied by many observers, posterior nuclear forms had not been described till Stephens and Fantham (1910) noted them in *T. rhodesiense*, the cause of a disease in man. This trypanosome is indistinguishable from *T. brucei*, and will be regarded as the human strain of this species. The question is more fully discussed below.

Distribution.—The polymorphic trypanosome which was named by Plimmer and Bradford (1899) is now known to be of wide distribution in Africa, extending from the Sudan to Zululand, though it has frequently been described under different names. It has been recorded from the

Sudan, Uganda, Rhodesia, East Africa, the territory around Lakes Nyasa and Tanganyika, and all the districts bordering on the Transvaal except those to the south. It will be seen that in West Africa this trypanosome is apparently absent, but a form morphologically indistinguishable from it was described from Togoland and the surrounding districts by Mesnil and Brimont (1909), and was named by them *T. togolense*. It was distinguished from *T. brucei* by immunity and inoculation tests. A trypanosome of much wider distribution in West Africa is *T. pecaudi* Laveran, 1907, which produces a disease known as baleri. Laveran and Mesnil describe this trypanosome as differing in certain respects from *T. brucei*, especially as regards the presence of certain small forms. The trypanosome which was described by Balfour (1909) and the writer (1909) in the Sudan as *T. pecaudi* is indistinguishable morphologically from *T. brucei*. Macfie (1913) described a trypanosome from Northern Nigeria which was indistinguishable from *T. brucei*, and the same form was isolated by Macfie (1914) by feeding wild *G. tachinoides* on guinea-pigs, so that it would appear that a trypanosome of the polymorphic type exists in animals all over tropical Africa. According to Laveran and Mesnil, *T. pecaudi* and *T. togolense* can be distinguished from *T. brucei* by their immunity reactions and other features. It is extremely doubtful, however, if any real specific differences exist between these forms. Similarly, the trypanosome which was isolated from a bush buck in the Luangwa Valley by Kinghorn and Yorke (1912*c*), and named by them *T. multiforme* on account of certain short forms of the *T. congolense* type, which were mixed with others of the *T. brucei* type, may in reality be *T. brucei*, or possibly a mixed infection of *T. brucei* and *T. congolense*. *T. suis*, described by Ochmann (1905) and Geisler (1912) from pigs in Somaliland, is probably *T. brucei*, as also *T. elephantis*, discovered by Bruce *et al.* (1909*b*) in an elephant of Uganda. Bruce *et al.* (1914*g*) discovered a trypanosome in three dogs in Nyasaland, which they regarded as an aberrant form of *T. brucei*. It produced a chronic type of infection instead of the usual acute one, and differed slightly in other respects from the usual *T. brucei* strains. It was inoculable to rabbits, dogs, and white rats, but not to oxen, goats, monkeys, or guinea-pigs. Though it was considered to be a modified form of *T. brucei*, the name *T. anceps* was suggested in case it should be decided to regard it as a new species.

Duke (1913) isolated a trypanosome of the *T. brucei* type from donkeys in East Africa. It was readily inoculable to most laboratory animals, but of seven guinea-pigs inoculated, only one became infected. The trypanosome was shown to develop in *G. palpalis* with salivary gland infection. Knuth and Du Toit (1921), for reasons which are not quite clear, propose to name this trypanosome *T. dukei*. There seems to be no

reason to regard it as other than *T. brucei*. For all practical and scientific purposes, the polymorphic trypanosome, which is highly virulent for small experimental animals, and which produces in these animals a varying percentage of posterior nuclear forms, may be regarded as *T. brucei*. There is, however, one difficulty in connection with *T. pecaudi*. Bouet and Roubaud (1910) studied the development of a trypanosome which they regarded as *T. pecaudi* in *G. tachinoides*, *G. longipalpis*, and *G. palpalis*. In the first they claim that the development commenced in the stomach, and was followed by infection of the proboscis only, the salivary glands not being involved. If this observation is correct, then there is a definite departure from what is known to occur in the case of *T. brucei*. Bouet and Roubaud's account of the development has not yet received confirmation, and as there is a possibility that they were not actually dealing with a polymorphic trypanosome, it is better to ignore it at present.

Susceptibility of Animals. — *T. brucei* is undoubtedly the most virulent of the known pathogenic trypanosomes. It is inoculable into all mammals, including monkeys, with the exception of the baboon (*Cynocephalus*), as noted by Bruce (1903). The latter animals enjoy an immunity, as do the majority of human beings. In the case of the last named it has long been known that travellers who have been constantly bitten by tsetse flies (*G. morsitans*), and who have lost all their transport animals through the ravages of these flies, have themselves remained perfectly healthy. The question of the immunity of man will be referred to again in connection with the relationship of *T. brucei* and *T. rhodesiense*.

Horses, mules, and donkeys are very susceptible, and die in a period varying from a fortnight to three months. For camels the strain is equally virulent. In the writer's experience, a convoy of over seventy camels taken into the Bahr el Ghazal province of the Sudan all died of the infection within a period of two months. Cattle, on the other hand, are not so rapidly killed as horses, but recoveries are rare. Sheep and goats are still less susceptible. Death may occur in four or five months, or few symptoms may be shown. Ultimately recovery may take place, with immunity to further infection. Pigs, on the other hand, quickly succumb. Dogs acquire the infection easily, and die in about a fortnight from the time of inoculation. Cats are slightly more tolerant. Rats and mice are easily infected. The former live for about a fortnight after inoculation, and mice for a shorter period. Sometimes, however, rats may survive for three weeks or more. Guinea-pigs live for three or four weeks. Monkeys, with the exception of the baboons, die of an intense infection in three weeks or a month. Many other animals are inoculable, and acquire infections similar to those indicated above. As a rule, the trypanosome

multiplies rapidly in the blood till near the end enormous numbers are present, and death may take place quite suddenly, suggesting a blockage of some important part of the circulatory system. It must not be forgotten that the virulence of any particular strain increases with passage through animals. Many laboratory strains have acquired a high virulence for rats, mice, and guinea-pigs after long maintenance in these animals. Some strains will kill mice in two or three days and rats in less than a week.

Hornby (1919*a*), in Rhodesia, has noted that if a convoy of horses and cattle are taken through fly areas, the general rule is for the horses or other equidæ to become infected with *T. brucei*, while the cattle acquire *T. congolense* and *T. vivax*. With few exceptions this rule seems to apply fairly regularly throughout the tsetse fly areas of Africa. It is possible therefore that nagana is a disease caused by several distinct trypanosomes.

As regards the susceptibility of birds, Mesnil and Martin (1906) showed that *T. brucei* could survive in geese for as long as three months, as proved by inoculation of blood into guinea-pigs. Durham (1908) had similar results in the case of an inoculated falcon (*Cerchneis tinnunculus*). Wendelstadt and Felmer (1909) showed that *T. brucei* could survive in the circulation of snakes and tortoises for about a week. Trypanosomes inoculated into the body cavity of two beetles survived seven and two days respectively, as proved by the inoculation of rats. Similarly in a snail the trypanosomes survived for two days.

Morphology.—*T. brucei*, being a polymorphic trypanosome, varies considerably in size (Plate V., B, p. 456). The measurements of a series given by Bruce (1911) are shown in the curve compared with *T. evansi* (Fig. 196). There occur the same three forms as are found in *T. gambiense*—namely, the short broad or stumpy form without a flagellum, the long slender one with a fairly long flagellum, and the intermediate form (Fig. 225). The short forms, however, tend to be broader than the corresponding ones of *T. gambiense*, and there is more variation in the position of the nucleus. In a certain number of broad forms, especially in infection in small laboratory animals, the nucleus becomes displaced towards the posterior end (posterior nuclear forms). Sometimes it may actually lie posterior to the kinetoplast. These posterior nuclear forms occur, not only in the undoubted *T. brucei*, but also in *T. pecaudi* and other West African strains, a fact which lends support to the view that they are identical. As will be seen below, they also occur in the human strain of *T. brucei* (*T. rhodesiense*), and afford a means of distinguishing this trypanosome from *T. gambiense*. It must be remembered, however, that it is only in the large infections seen in rats, mice, and guinea-pigs that the characteristic posterior nuclear forms are met with to any extent. The

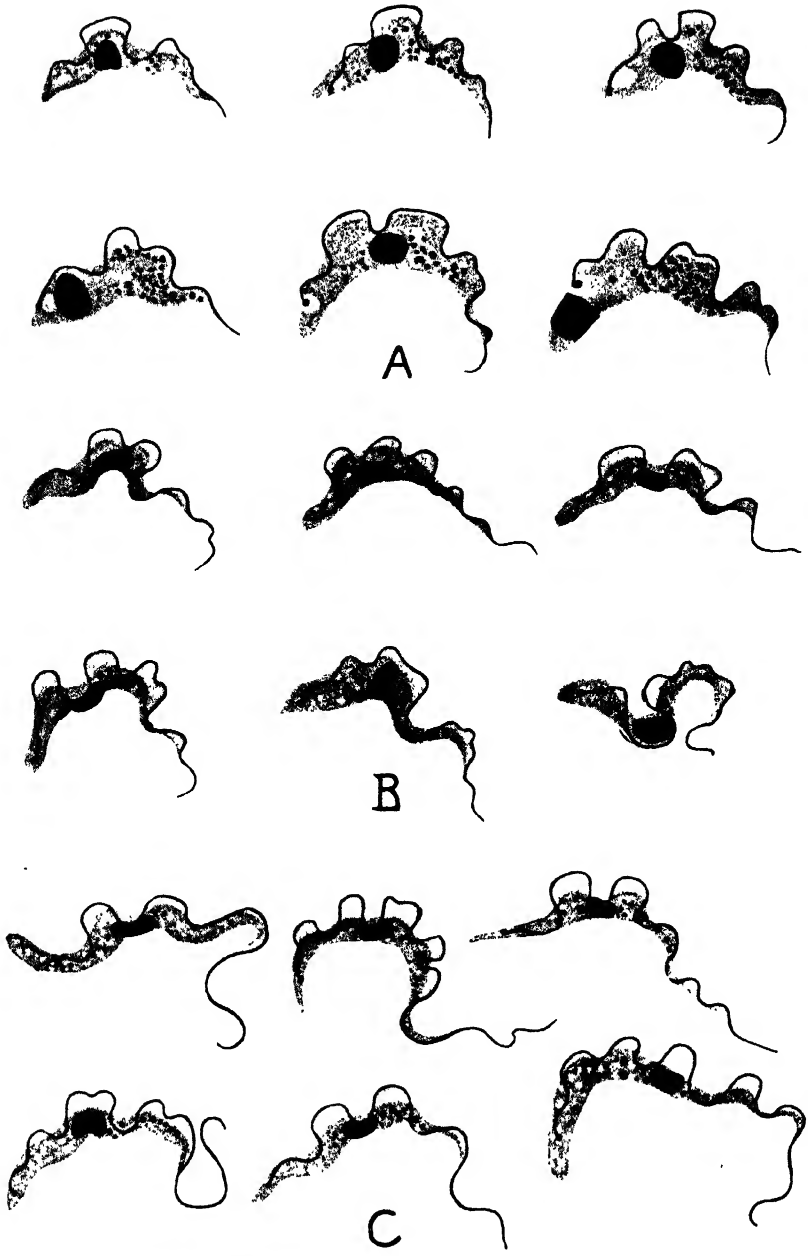


FIG. 225.—*Trypanosoma brucei* (×2,000). (AFTER BRUCE, HARVEY, HAMERTON, AND LADY BRUCE, 1914.)

A. Broad stumpy form; two posterior nuclear forms are shown, one with kinetoplast behind the nucleus, and one with it in front.
B. Intermediate form with short flagellum.
C. Long slender form with flagellum.

measurements of *T. brucei* (Zululand strain) in inoculated animals are given by Bruce and his co-workers (1914) as follows:

Species of Animal.	*Number of Trypanosomes Measured.*	*Measurement in Microns.*		
		Average Length.	*Maximum Length.*	*Minimum Length.*
Monkey	160	21·2	31·0	12·0
Dog	260	21·5	32·0	16·0
Guinea-pig	30	22·9	35·0	17·0
Rat	500	20·8	28·0	17·0

A curve showing the percentage of trypanosomes of various lengths is given in Fig. 196. The percentage of posterior nuclear forms varies in different strains. In the case of an infection in rats of the Sudan strain, thirty-six trypanosomes out of 1,138 were found by the writer (1912) to show this posterior displacement of the nucleus.

Transmission.—The transmission of *T. brucei* by the tsetse fly was demonstrated by Bruce (1897) in Zululand. At that time Bruce considered the fly to be *G. morsitans*, but from observations made later, Austen (1903) came to the conclusion that the fly with which Bruce worked was probably *G. pallidipes*. It was Kleine (1909*a*) who first demonstrated that flies did not become infective till a period of eighteen to twenty days had expired from the time of feeding. The experiments were made by feeding *G. palpalis* on sheep and a mule previously infected with *T. brucei* by the bites of *G. morsitans*. The flies were then fed each day on a healthy animal, and it was found that the only animals to become infected were those bitten after the long incubation period. Bouet and Roubaud (1910), working with the same trypanosome (*T. pecaudi*) in West Africa, found that *G. longipalpis*, *G. tachinoides*, and *G. palpalis* were all capable of transmitting the trypanosome, though the former was the most frequent carrier and the latter only rarely so. They further found that the same trypanosome was carried by *G. morsitans*. Macfie (1914) and Gallagher (1914) obtained strains of *T. brucei* by feeding wild *G. tachinoides* on guinea-pigs in the Eket district of Nigeria. Transmission by means of *G. tachinoides* has also been effected by Lloyd and Johnson (1924). Bruce *et al.* (1913, 1913*a*), working in Nyasaland, found that *G. morsitans* was the usual carrier, but they also noted (1914*b*) that *G. brevipalpis* could serve as a vector, both with the Nyasaland strain and that from Zululand.

Cycle in the Tsetse Fly.——The developmental cycle of *T. brucei* in *G. morsitans*, as demonstrated by Bruce *et al.* (1914*a*, 1914*h*, 1914*i*), follows

very closely that of *T. gambiense* in *G. palpalis* as described above (Figs. 217 and 223). There is an intestinal phase followed by migration forwards of long thin trypanosomes to the proventriculus, labial cavity, and hypopharynx, and finally to the salivary gland, where, after the production of free and attached crithidia forms, the infective metacyclic trypanosomes arise (Fig. 226). The characters of the developmental forms as described by Lloyd and Johnson (1924) have been referred to above (p. 515). They

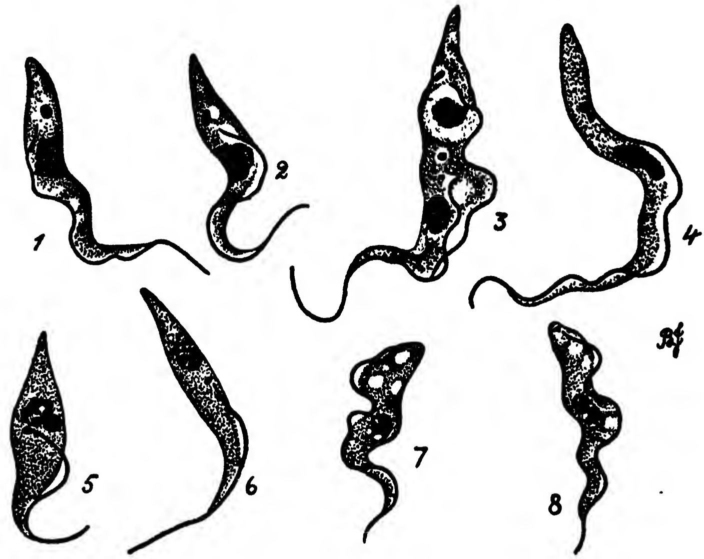


FIG. 226.—DEVELOPMENTAL FORM OF *Trypanosoma brucei* IN *Glossina tachinoides* (×2,000). (AFTER LLOYD AND JOHNSON, 1924.)

1-3. Forms in mid-gut. 4. Form in proventriculus.
5-6. Crithidia forms from salivary glands. 7-8. Infective forms from salivary glands.

are so similar to the developmental forms of *T. gambiense* that it is impossible to differentiate the two trypanosomes as they occur in the fly. Lloyd and Johnson give the dimensions in microns of the infective forms as they occur in the salivary glands of *G. tachinoides* as follows:

	Average Length.	*Average Length of Flagellum.*	*Average Breadth at Nucleus.*
T. brucei	15·8 (13·3–18·0)	2·1 (1·1–3·5)	2·2 (1·4–3·0)
T. gambiense	14·6 (12·1–17·3)	1·7 (0·5–2·8)	1·5 (1·0–2·5)

Reservoir Hosts.—In his investigations in Zululand, Bruce (1895) found that the wild *G. morsitans* readily infected dogs and other animals. As there were no domestic animals alive in the district, it was evident that

another source of infection existed. The wild fauna was examined, and it was discovered that 24 per cent. harboured trypanosomes. In later investigations in Nyasaland, Bruce *et al.* (1913*e*) found that as many as 31·7 per cent. of the wild game harboured *T. brucei* or other species pathogenic for domestic animals. Similar results were obtained by Kinghorn and Yorke (1912*a*) in North-East Rhodesia, but they wrote of the trypanosome as *T. rhodesiense,* which, however, they regarded as identical with *T. brucei.* The wild game do not appear to be seriously affected by their infections, and it is evident that they form a reservoir for the virus, which is transmitted to domestic animals by the tsetse flies (see p. 508). On account of the wild fauna, development of these countries is handicapped to such an extent that some have advocated the complete extermination of the game. If, as seems probable, *T. rhodesiense* is identical with *T. brucei,* then the question is a still more important one.

The Human Strain of Trypanosoma brucei.

Trypanosoma rhodesiense Stephens and Fantham, 1910.—This trypanosome, which produces a disease in man differing in many respects from that caused by *T. gambiense,* was first recognized as distinct from the latter by Stephens and Fantham (1910). The chief feature not shown by *T. gambiense* is the presence of posterior nuclear forms in small laboratory animals inoculated from man. The disease in man is of a more serious type than that produced by *T. gambiense,* and runs a course of only a few months. It is only exceptionally that the symptoms characteristic of sleeping sickness appear. The disease is too rapidly fatal to allow of the changes in the central nervous system which occur in the more chronic infections with *T. gambiense.*

Distribution.—The disease in man has a very restricted distribution when compared with sleeping sickness due to *T. gambiense.* It is limited to the districts east and west of Lake Nyasa, and occurs in Northern Rhodesia, Nyasaland, the south-east corner of Tanganyika Territory, and the north-east part of Mozambique. Cases have also been recorded from South Rhodesia near Livingstone. Outside this area, in which *T. gambiense* infections do not occur, there are only two records of the occurrence of the infection. Duke (1923) studied an epidemic at Mwanza in the district bordering the south-east corner of Victoria Nyanza, while Archibald (1922) isolated from sleeping sickness cases in the Southern Sudan a trypanosome which corresponded morphologically with *T. brucei.* Though *T. gambiense* infections occur at the north end of Lake Victoria, they have not been recorded from Mwanza, the only district where the two infections appear to overlap being in the Southern Sudan.

Hearsey (1909) was the first to report cases of human trypanosomiasis from districts in which *G. palpalis* was not known to occur, and to suspect the existence of a disease distinct from the well-known sleeping sickness.

Relation to T. brucei of Animals and T. gambiense of Man.—Though the characters of the trypanosome as described by Stephens and Fantham serve to distinguish it from *T. gambiense*, this is not so for *T. brucei* in animals, which it resembles so closely as to be morphologically indistinguishable. The difficulty in dealing with this trypanosome is that there is a divergence of opinion as to whether it is distinct from *T. brucei* or not. Bruce and his co-workers (1913*e*) in Nyasaland came to the conclusion that no differences exist between the trypanosomes producing disease in man and animals, and wrote of it as *T. brucei* vel *rhodesiense*. The trypanosome of Nyasaland was also found to be identical with a strain of *T. brucei* from Zululand, from which country the original *T. brucei* came. This similarity applies to all stages of the organism, whether in man, wild game, experimental animals, or tsetse flies, so that there seems no reason to regard *T. rhodesiense* as being distinct from *T. brucei*. Kinghorn and Yorke (1912) arrived at the same conclusion in Rhodesia. It is, however, a well-known fact that in many localities where nagana is widespread amongst animals, and where human beings are constantly bitten by *G. morsitans* which are actually transmitting *T. brucei* to animals, no cases of human infection have been recorded. Moreover, in those districts in which trypanosomes of this type produce disease in both man and animals the number of human cases is much lower than those in animals. Some observers, as, for instance, Taute (1913), Kleine (1914), Beck (1914), believe that the human cases in these areas are due to a distinct trypanosome, *T. rhodesiense*, and the animal cases to *T. brucei*. Kleine (1923) again makes this assertion, and concludes that an animal reservoir of *T. rhodesiense* is unknown. Those who regard the trypanosomes as identical suppose that man is much less susceptible to infection than animals. Furthermore, it has been suggested that this type of human trypanosomiasis is a new disease, and there is some evidence in support of this view. It is conceivable that it was only in one area that *T. brucei* became capable of infecting man, and that, having once acquired this property, the particular strain has now commenced to extend to other areas.

Taute believes the two trypanosomes are distinct, not only for the reasons given above, but from the results of a series of experiments conducted by himself and Huber (1919). A large number of human beings, natives of Africa belonging to different tribes, to the number of 129, and also the two observers themselves, were inoculated with *T. brucei* derived from four horses and two mules which were discovered naturally

infected. In no case did an infection result. In an earlier experiment Taute fed infected *G. morsitans* on animals and on himself. He was immune, but all the animals acquired the disease. These experiments, at any rate, prove that man is not easily inoculated with *T. brucei*, though they do not conclusively prove that he never can be. Kleine (1923) further maintains that the only means of distinguishing *T. brucei* from *T. rhodesiense* in naturally infected flies and animals is to test the susceptibility of human beings, as was actually done in the experiment mentioned above. If this view is correct, it becomes practically impossible to distinguish them. To the writer, however, it seems that the evidence at present available is in favour of the identity of the human and animal strains. The animal strain (*T. brucei*) is not readily inoculable to man, but once having gained a footing there, it is more easily passed to other men. In attempting to isolate *T. equiperdum* from horses, Watson only succeeded in inoculating the trypanosome to a laboratory animal after many hundred failures. Directly this was accomplished, the blood of this animal readily infected other laboratory animals, and even horses, so that the strain was easily maintained.

It seems probable that *T. gambiense* also originated from *T. brucei* of animals, it may be centuries ago, and that having passed from man to man through many passages, has become modified morphologically (disappearance of posterior nuclear forms) and as regards its virulence for laboratory animals. The human strain of *T. brucei*, on the other hand, represents the animal strain which has only recently infected man, and, having been subjected to few passages, still maintains its morphological characters and virulence. *T. gambiense* is sufficiently distinct to be regarded as a species, but *T. rhodesiense* is merely a strain of *T. brucei* in man.

Duke (1921, 1923*a*) has expressed a similar view, but suggests that *T. gambiense* and *T. brucei* are still more nearly related. He points out that his previous investigations (1912*c*) of the trypanosomes occurring in the Sesse Islands of Victoria Nyanza before the population was removed revealed only *T. gambiense* in man and a similar trypanosome in the sitatunga. He reinvestigated the subject over ten years after the islands were depopulated. He finds that *G. palpalis*, no longer having human beings to feed upon, nourishes itself on the sitatunga, which have increased considerably in numbers. The trypanosome now isolated from these animals is of the *T. brucei* type, and Duke believes that the trypanosomes of the *T. gambiense* type, which originally were handed on in a mechanical manner from man to man by the flies, have, since the depopulation, been handed from sitatunga to sitatunga by the same flies, which have been driven to feed on them exclusively, with a consequent

reversion of the trypanosome to its antelope type. As pointed out above (p. 539), the possibility of the occurrence of posterior nuclear forms in small laboratory animals was not excluded in Duke's investigations of 1912.

It is well known that *G. morsitans*, the chief carrier of *T. brucei*, lives a considerable distance from water, while *G. palpalis*, the vector of *T. gambiense*, is found along the water-courses or near the lake shores. The former is sometimes spoken of as the "dry fly," and the latter as the "wet one." It may be supposed that when *T. brucei* first gained entrance to human beings, who naturally live near to water, it was *G. palpalis* which necessarily passed the infection from man to man. Antelope, on the other hand, spend the daytime in districts far from water, where *G. morsitans* is found, and travel long distances at night to drink. In this manner it may be supposed that the human strain gradually became adapted to *G. palpalis*, while the animal strain remained in association with *G. morsitans*. If this be the case, there would be a double chance of the trypanosome becoming modified morphologically. Duke (1923) described an epidemic of trypanosomiasis east of Mwanza in the Tanganyika Territory (lat. 5° to 2° 3′ S., long. 33° 30′ to 34°), which throws light on the question under discussion. The trypanosome causing the disease was of the *T. brucei* type when inoculated to small animals. Duke believes the human infection arose in 1919 during a famine and an influenza outbreak which reduced the resistance of the already ankylostome-ridden population, and made them susceptible to infection with *T. brucei*, which occurred in the game. He does not think the outbreak was due to imported infection. The vector in this locality is the recently discovered *G. swynnertoni*. Duke believes that the epidemic was due to mechanical transmission from man to man, in spite of the fact that wild flies, infected from either game or man, were discovered in this locality. The cyclically infected flies, including those infected from man, he regards as responsible for the spread of infection amongst the game, man not being susceptible even when the flies had a salivary gland infection derived from man. According to this hypothesis, a fly with a salivary gland infection acquired from a feed on an infected human being would not necessarily be capable of infecting another human being, though it would certainly infect game. In the writer's opinion very substantial evidence is required before this view can be accepted.

It has still to be mentioned that Laveran and Mesnil (1912) separate *T. rhodesiense* as a distinct species from *T. gambiense* and *T. brucei*, as a result of cross-immunity and serological tests carried out chiefly by Mesnil and Ringenbach (1911*a*, 1912*b*), Laveran (1911*a*, 1912, 1912*a*), and Laveran and Nattan-Larrier (1912, 1912*b*).

Morphology.—As already remarked, the human strain of *T. brucei* is morphologically indistinguishable from that derived from animals, so that Fig. 225, illustrating the latter, will apply equally well to the human strain. In the inoculated small animals the posterior nuclear forms appear. The number of these, however, varies considerably. Thus, in three strains isolated from men and investigated by the writer and Hanschell (1913) the percentages in rats examined on different days varied between 0 and 9·3, 0 and 7·2, and 13 and 40. These observations were made in a series of rats, in which 1,000 trypanosomes were counted every three days. After long passage through rats, the number of posterior nuclear forms may diminish considerably till they become difficult to find.

Susceptibility of Animals.—In its effect on animals, *T. rhodesiense* does not differ in any way from *T. brucei.* It is readily inoculable from man to laboratory animals. If a rat is inoculated with even a few drops of blood from a human case, it quickly acquires a large infection. The trypanosome is virulent to rats from the start, and in this respect differs from *T. gambiense.* A rat inoculated directly from a man with *T. gambiense* acquires a very chronic type of infection, during which trypanosomes are rarely numerous in the blood. It is only after many passages through rats that *T. gambiense* attains anything like the virulence the human strain of *T. brucei* has at its first passage into laboratory animals.

Transmission.—The human strain of *T. brucei* was proved to be conveyed by *G. morsitans* by Kinghorn and Yorke (1912) in Northern Rhodesia. These observers found that the percentage of wild flies infected varied with the altitude. In a valley (2,100 feet, temperature 75° to 83°) 1 in 534 flies was found naturally infected, whereas on the plateau (4,400 feet), with a mean temperature 15° to 20° lower than in the valley, only 1 in 1,260 was found infected. By actual feeding experiments in the valley, about 3 per cent. of flies became infective. Flies fed and kept at the lower temperature did not become infective, though it was shown that after a period of sixty days at the lower temperature the flies became infective when the temperature was raised. The low temperature is thus compatible with the early stages of development, but the final stage requires a higher one.

Bruce and his co-workers (1913, 1914*b*) in Nyasaland found that *G. morsitans* was the principal agent, but *G. brevipalpis* was also incriminated. The course of development in the fly is identical with that of *T. gambiense* and the animal strains of *T. brucei.*

Reservoir.—As regards the reservoir hosts, Bruce and his co-workers found that in Nyasaland a large proportion of the wild game harboured the trypanosome, and Kinghorn and Yorke (1912) found the same state of affairs in Northern Rhodesia They, like Bruce, found a natural

infection in the dog. From what has been stated above, it is evident that there are no means of distinguishing the trypanosomes which occur naturally in game and tsetse flies from the form found in man. Kleine (1923), who regards the human strain as a species (*T. rhodesiense*) distinct from *T. brucei*, maintains that, in spite of morphological identity, the forms seen in the wild animals by Bruce and his co-workers and by Kinghorn and Yorke are *T. brucei*, and that a true reservoir for the human trypanosome, *T. rhodesiense*, has yet to be discovered.

(*b*) Trypanosomes which Develop in the Stomach and Proboscis of Tsetse Flies—Monomorphic Trypanosomes without Flagella.

Trypanosoma congolense Broden, 1904.—Synonyms: *T. dimorphon* Laveran and Mesnil, 1904 *pro parte;* *T. nanum* Laveran, 1905; *Trypanozoon dimorphon* (Lühe, 1906) *pro parte;* *T. nanum* (Lühe, 1906); *T. congolense* (Lühe, 1906); *Trypanosoma confusum* Montgomery and Kinghorn, 1909; *T. pecorum* Bruce *et al.*, 1910; *T. somaliense* Martoglio, 1911; *T. cellii* Martoglio, 1911; *T. frobeniusi* Weissenborn, 1911; *Duttonella pecorum* (Chalmers, 1918); *T. montgomeryi* Laveran, 1909 (?); *T. ruandæ* van Saceghem, 1921.

Distribution.—This is a small trypanosome found chiefly in cattle, but also in horses and sheep. It was first recorded by Broden (1904) in the Congo. What was probably the same form was discovered by Balfour and Head in the Sudan, and was named *T. nanum* by Laveran (1905*d*). Montgomery and Kinghorn (1909) suggested the name *T. confusum* for this trypanosome on account of the doubt as to its identity. Finally, Bruce *et al.* (1910*b*), because of the same confusion, proposed to start *de novo* with the name *T. pecorum*. There seems little doubt that all these forms are identical, in spite of certain differences as regards the susceptibility of small laboratory animals. *T. nanum*, for instance, as noted by Bruce *et al.* (1911*b*), is said to be not inoculable into rats and mice, whereas the form named *T. pecorum* could be often transmitted to these animals. Bruce (1914), however, stated that after passage through the goat, *T. pecorum* ceased to infect rats and mice, and came to the conclusion that *T. nanum* was merely a strain of *T. pecorum* which had lost its virulence. Aders (1923) in Zanzibar has noted the same loss of virulence after passage through the goat, sheep, and giant rat. Morphologically, the various forms are indistinguishable, and it seems safer to regard the small pathogenic trypanosomes which are widely distributed in Africa as belonging to one species. There is a greater difficulty, however, with a form which was named *T. dimorphon* by Laveran and Mesnil (1904). This trypanosome was first seen by Dutton and Todd in the Gambia in 1903, and the strain brought home was sent to Laveran and Mesnil, who have employed it in a long series of investigations. They maintain (1912) that it is distinct from any of the forms mentioned above, not only because of cross-

immunity tests, but also on account of the fact that in infected animals a small percentage of the trypanosomes present measure up to 25 microns in length, while the remainder are small forms like *T. congolense.* It must be admitted, however, that the trypanosome was isolated in the early days of trypanosome investigations, and that the possibility of mixed infections was not then considered. Yorke and Blacklock (1911), from the examination of two naturally infected horses in the Gambia, came to the conclusion that the original *T. dimorphon* strain was a mixed one of

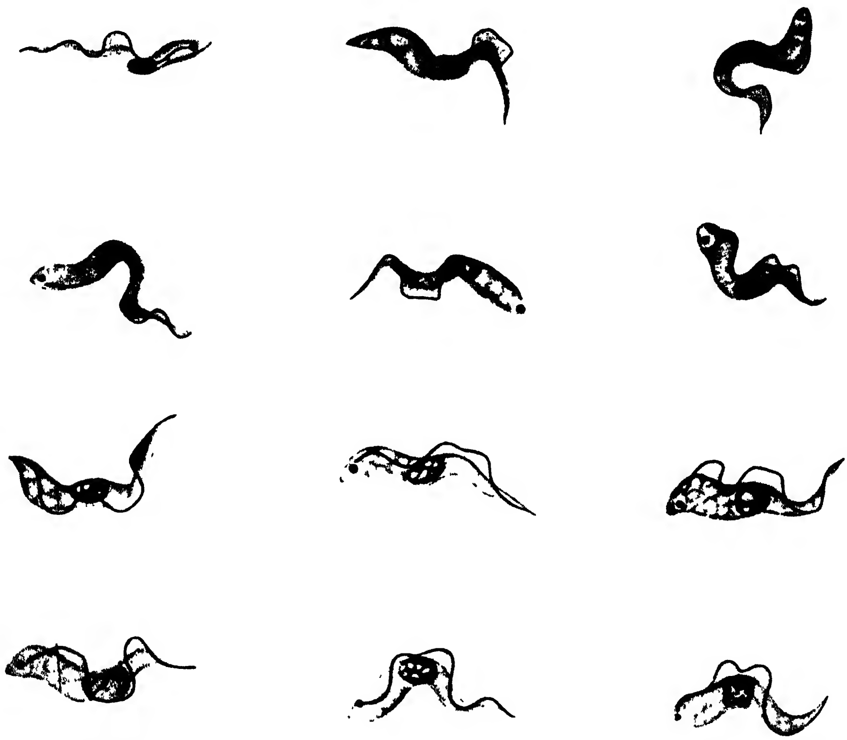


FIG. 227.—*Trypanosoma congolense* (×2,000). (AFTER BRUCE, HAMERTON, BATEMAN, MACKIE, AND LADY BRUCE, 1910 AND 1911.)

T. congolense and *T. vivax.* Whichever view may be correct, it is doubtful if the exact counterpart of the original strain has been rediscovered since, though French writers have frequently employed the name *T. dimorphon* for the trypanosome of the *T. congolense* type, while others have used it for one of the *T. brucei* type. The small pathogenic trypanosome of wide distribution in Africa should therefore be known by Broden's name, *T. congolense.*

Morphology.—*T. congolense* is the smallest of the pathogenic African trypanosomes, and varies in length from 9 to 18 microns, with an average

of 14 microns (Fig. 227, Plate V., H, p. 456). Its breadth is under 3 microns. There are no forms with a flagellum, though sometimes in certain individuals there may be difficulty in deciding whether a short one is present or not. The nucleus is central in position, while the kinetoplast often projects over the border of the parasite. According to Laveran and Mesnil (1912), *T. dimorphon* occasionally shows much larger forms up to 20 or even 25 microns in length, but the majority of the trypanosomes in any pure infection of *T. congolense* fall within the dimensions given above.

The trypanosome occurs naturally in horses, donkeys, oxen, goats, sheep, pigs, and dogs, in which it produces a rather chronic wasting disease associated with fever and progressive anæmia. Rats may sometimes be inoculated, but, as pointed out by Bruce and his co-workers (1913*b*), many strains have no effect on rats.

Working with what was undoubtedly this trypanosome in the Sudan, the writer produced an infection in two dogs which he had inoculated from cattle.

Transmission and Reservoir.—The wild game of Nyasaland were shown by Bruce *et al.* (1913*e*) to be reservoirs of *T. congolense*, as many as 14·4 per cent. of those examined in the "fly country" being found infected (see p. 509). It was further shown (1914*c*) that the strain with which they worked (*T. pecorum*) was conveyed by *Glossina morsitans.* Bouet and Roubaud (1910), with the trypanosome they called *T. dimorphon*, found that *G. longipalpis* was the chief carrier, but that *G. tachinoides* and *G. palpalis* could also act as vectors. Bruce *et al.* (1910*a*) and Fraser and Duke (1912*c*) in Uganda found that the strain (*T. pecorum*) was carried by *G. palpalis*, while Bruce *et al.* (1914*b*) found that *G. brevipalpis* could transmit the trypanosome in Nyasaland. Croveri (1919) showed that in Somaliland *G. pallidipes* was the vector of this trypanosome, which was referred to as *T. somaliense.* Duke (1923*c*) has again effected the transmission of *T. congolense* by *G. palpalis* in Uganda.

Cycle in the Tsetse Fly.—The mode of development in the tsetse fly, which differs from that of *T. gambiense*, was studied by Robertson (1913) and Bruce *et al.* (1914*c*). There is at first an intestinal development, followed by migration forwards of long narrow trypanosomes to the hypopharynx, where a change into attached crithidia forms and then into trypanosomes of the blood type takes place, after which the flies are infective (Fig. 218). There is no invasion of the salivary glands, as there is with *T. gambiense.* Duke (1912) in Uganda found that the part of the proboscis which became most heavily infected was the labrum, while in only one instance were trypanosomes observed in small numbers in the hypopharynx. After the forward migration of the intestinal forms,

a change into crithidia forms takes place, and these attach themselves to the inner surface of the labrum, just as they do in the salivary glands in the case of *T. gambiense.* It is from these crithidia forms that the final infective metacyclic trypanosomes are evolved.

Lloyd and Johnson (1924) find that during the early stages of development the forms in the gut are short trypanosomes which are feebly undulant and have no free flagellum (Fig. 228). There are then produced long trypanosomes with free flagella, which migrate to the proventriculus and thence to the labial cavity. These are difficult to distinguish from

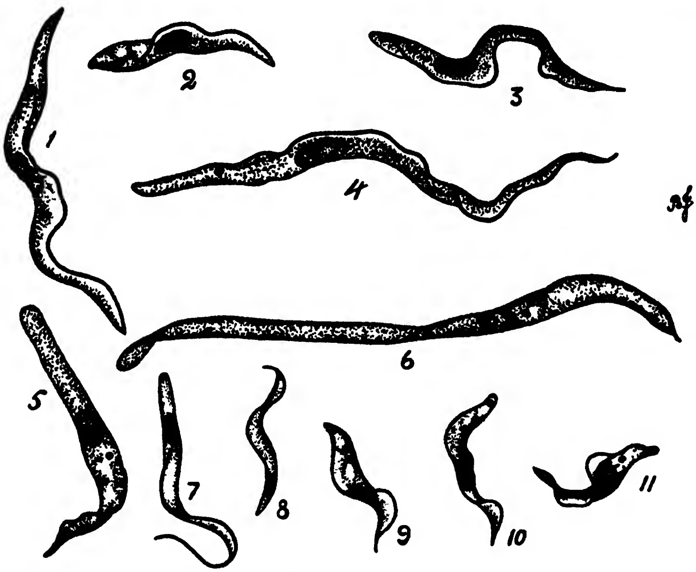


FIG 228.—DEVELOPMENTAL FORMS OF *T. congolense* IN *Glossina tachinoides* (×2,000). (AFTER LLOYD AND JOHNSON, 1924.)

1-3. Forms in mid-gut. 4. Form in proventriculus.
5-6. Forms from fixed colonies in labial cavity.
7-8. Pre-infective forms from labial cavity in hypopharynx.
9-10. Infective forms from hypopharynx.
11. Infective form from hypopharynx of *Glossina morsitans.*

the corresponding forms of *T. gambiense* or *T. brucei.* In the labial cavity they become crithidia forms without flagella, and are attached in compact colonies. The corresponding stages of *T. vivax* have flagella. Subsequently slender crithidia forms, with the nuclei and kinetoplasts close together at the posterior end of the body and with flagella, are produced. These invade the hypopharynx and give rise to infective forms, which resemble the trypanosomes of the blood in that there are no flagella. In contrast to *T. vivax* infections the metacyclic trypanosomes are numerous in the hypopharynx.

A series of measurements of the infective forms from the hypopharynx of three species of tsetse fly gave the following average dimensions:

	Length.	Breadth at Nucleus.
Glossina tachinoides	11·1	1·6
Glossina palpalis	11·1	1·7
Glossina morsitans	11·2	1·6

By attention to the features detailed above, Lloyd and Johnson claim that a *T. congolense* infection can be recognized in tsetse flies (see p. 514).

It is possible that *T. montgomeryi* Laveran, 1909, seen once by Montgomery and Kinghorn (1909) in Rhodesian cows, and *T. somaliense* and *T. cellii*, described by Martoglio (1911) as the cause of disease in cattle, horses, sheep, and camels in Somaliland, belong to the *T. congolense* group. The same remark applies to *T. frobeniusi*, discovered by Weissenborn (1911) in Hamburg in the blood of a horse which had been brought there from Togoland. *T. montgomeryi* or a very similar form was again seen

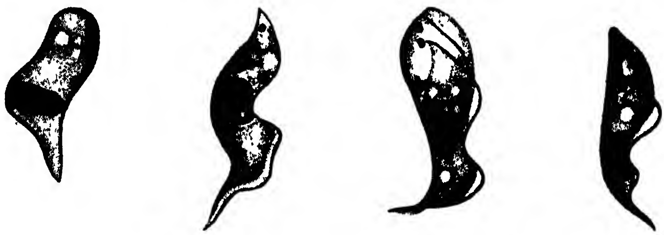


FIG. 229.—*Trypanosoma montgomeryi* FROM BLOOD OF NYASALAND DOG (×2,000). (AFTER KINGHORN AND YORKE, 1913.)

by Kinghorn and Yorke (1912*a*) in a dog in Rhodesia, and, as it is distinctly broader than *T. congolense* (1·25 to 6·5 microns), it may be a separate species (Fig. 229). Lloyd has, however, shown the writer a slide of undoubted *T. congolense* from a sheep in which numerous forms apparently identical with *T. montgomeryi* occur. The trypanosome found by Edington (1908) in a horse in Zanzibar is probably *T. congolense*. Writing of this trypanosome, Aders (1923) notes that with the importation of cattle from Africa there are introduced, not only *T. congolense*, but also *T. brucei*, *T. vivax*, and a trypanosome resembling *T. evansi*. Of these, only *T. congolense* has established itself in the island, and this has taken place in the absence of tsetse flies. Tabanids and possibly other biting flies appear to be the vectors.

With reference to *T. somaliense* and *T. cellii*, Donizio (1921) has reinvestigated the trypanosomes of Italian Somaliland, and has found that two forms exist—one, of the *T. brucei* type, affecting chiefly equidæ, and the other, of the *T. congolense* type, producing disease in cattle. He concludes

with ample justification that Martoglio was probably dealing with one or both of these trypanosomes in pure or mixed infections.

Stirling (1921) found a trypanosome in large numbers in the blood of a bullock which had died in the Central Provinces of India. The trypanosome was quite unlike *T. evansi*, measured 11 to 18 microns in length, with an average of 14·5 microns, and had the characters of *T. congolense*. Stirling concluded that it was actually *T. congolense*, of which this is the first record outside Africa. In such a case there might have been some grounds for the creation of a new species, but there are none whatever for the name *T. ruandæ* given by Van Saceghem (1921) to a trypanosome of the Belgian Congo which is undoubtedly *T. congolense*.

Trypanosoma simiæ Bruce *et al.* 1912.—Synonyms: *T. ignotum* Kinghorn and Yorke, 1912; *Duttonella simiæ* (Chalmers, 1918).

This is a trypanosome which was discovered by Bruce *et al.* (1912) in Nyasaland. What was probably the same trypanosome was also seen later by Kinghorn and Yorke (1912*b*) in Rhodesia. The latter observers

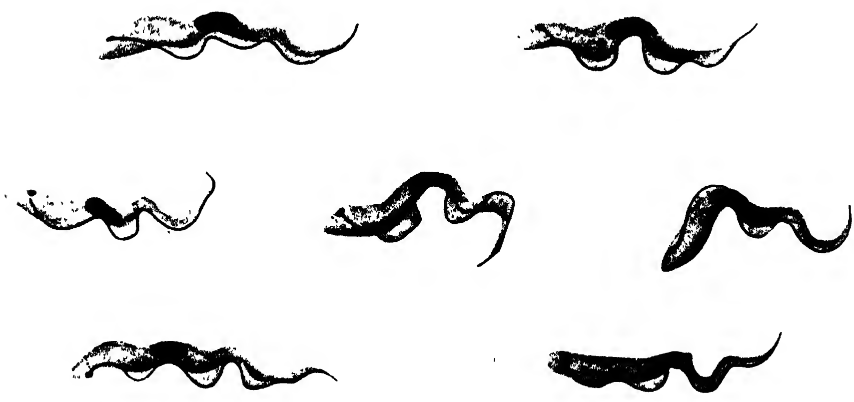


FIG. 230.—*Trypanosoma simiæ* FROM BLOOD OF MONKEY (×2,000). (AFTER BRUCE, HARVEY, HAMERTON, AND LADY BRUCE, 1914.)

isolated it by feeding wild *G. morsitans* on monkeys in the Luangwa Valley, and, being unaware that it had already been named, called it *T. ignotum*.

Morphologically *T. simiæ* resembles *T. congolense*, which has been considered above, except that it is distinctly larger (Fig. 230, Plate V., G, p. 456). Bruce *et al.* (1913*d*) found that the trypanosome was remarkable for its virulence to the monkey and domestic pig. Goats and sheep are also susceptible, but other animals, including rats, mice, and guinea-pigs, are refractory. It was noted that when a monkey and a goat are exposed to bites of infected flies, both acquire an infection, but the monkey in such

an acute form that death takes place in a few days. The infection in the goat is of a chronic nature, and the animal may recover. If, however, a monkey is inoculated from the goat, as a rule no infection takes place, an experiment which proves that passage through the goat may profoundly modify the virulence of a trypanosome. The loss of virulence of *T. pecorum* for the rat after passage through the goat is another instance of the same change. These observations are of considerable interest in throwing light on the real value of inoculation tests as a means of separating trypanosomes generally.

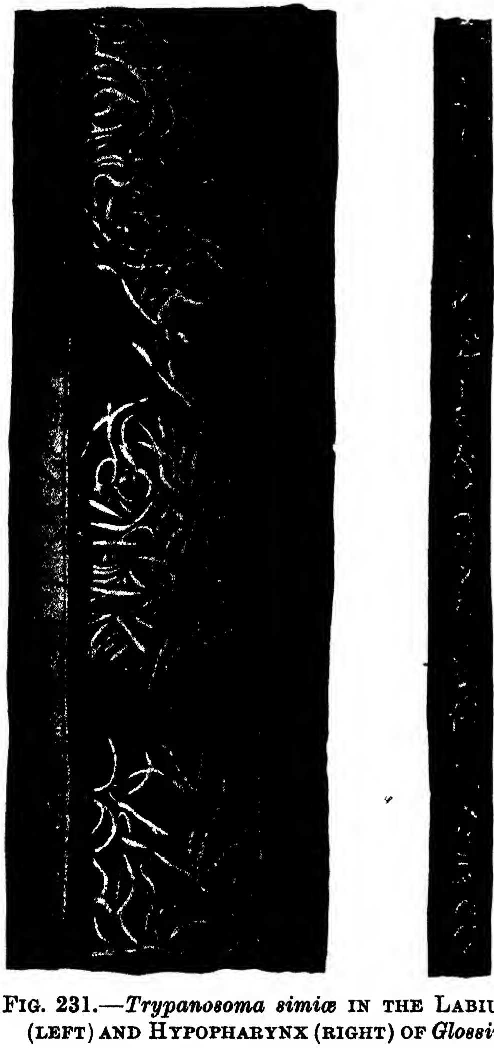


FIG. 231.—*Trypanosoma simiæ* IN THE LABIUM (LEFT) AND HYPOPHARYNX (RIGHT) OF *Glossina morsitans* (× 520). (AFTER BRUCE, HARVEY, HAMERTON, AND LADY BRUCE, 1913.)

Morphology.—*T. simiæ* varies in length from 14 to 24 microns; the majority of forms measure about 18 microns, and are thus larger than those of *T. congolense*, which have an average length of only 14 microns (Fig. 230). There is no flagellum, but in some individuals there is a difficulty, as occurs also with *T. congolense*, in deciding whether the last few microns represent a flagellum or not. The nucleus is central, while the kinetoplast is at the margin of the trypanosome, about 1·5 microns from the posterior end, which is generally more or less rounded. As suggested by Hornby (1921), it is possible that *T. simiæ* is merely a race of *T. congolense* modified by passage through the wart hog.

Transmission and Cycle in Tsetse Fly.—The transmission of *T. simiæ* by *Glossina morsitans* was demonstrated by Bruce *et al.* (1912) in Nyasaland,

an observation made independently but shortly after by Kinghorn and Yorke (1912*b*) in the Luangwa Valley. Bruce *et al.* (1914*b*) found that *G. brevipalpis* might be naturally infected with this trypanosome. They also showed (1913*d*) that the wart hog (*Phacochœrus æthiopicus*) was the natural reservoir. They found that the course of development in the fly was similar to that of *T. congolense*, the usual period of about twenty days being required before the fly becomes infective. The development commences in the stomach. Finally, the labial cavity is infected, where crithidia forms are evolved. These invade the hypopharynx and develop into metacyclic trypanosomes (Fig. 231).

(*c*) Trypanosomes which Develop only in the Proboscis of Tsetse Flies—Monomorphic Trypanosomes provided with Flagella.

Trypanosoma vivax Ziemann, 1905.—Synonyms: *T. cazalboui* Laveran, 1906; *Trypanozoon vivax* (Lühe, 1906); *Trypanosoma bovis* Kleine, 1910; *T. angolense* Broden and Rodhain, 1910; *Duttonella vivax* (Chalmers, 1916).

As with so many of the pathogenic trypanosomes, there has been considerable confusion over the correct name of *Trypanosoma vivax.* Ziemann (1905) described a very active trypanosome from the blood of cattle, sheep, and goats in the Cameroons. It was of wide distribution, and was seen many times in the area he investigated. With blood taken from infected animals a series of inoculations was made. Eight grey rats which had suffered from *T. lewisi* infection were inoculated, and died in eight to eleven days. One white rat was inoculated, but did not become infected, nor did a cat. A dog showed scanty trypanosomes after ten days, but these quickly disappeared, and did not recur. Laveran (1906) gave the name *T. cazalboui* to a very similar trypanosome which Cazalbou had studied in cattle in the Upper Niger region. This trypanosome, though inoculable to sheep and goats, was not inoculable to monkeys, dogs, guinea-pigs, rats, and mice.

The question is whether this form is identical with Ziemann's *T. vivax.* If it be regarded as identical, it has to be explained how Ziemann infected his eight rats, for all subsequent workers are agreed that the trypanosome of this type is not inoculable to these animals, and it was chiefly for this reason that the new species, *T. cazalboui*, was created. It is just possible that Ziemann mistook *T. lewisi* in the rats for *T. vivax*, for he states earlier in his paper that when *T. brucei* and *T. lewisi* exist together in the rat, they are easily distinguished, whereas with *T. vivax* and *T. lewisi* this may be very difficult unless stained films are examined, and no details of the infection in the rats are given.

That the eight rats died is again explicable from the fact that wild rats frequently die in captivity quite apart from any infection. Whether this will explain the discrepancy or not, it may be noted that in the other inoculations—namely, the white rat, the cat, and the dog—only a slight transitory infection took place in the dog, and this is in agreement with all later observations on *T. vivax.* Laveran and Mesnil (1912) state very emphatically that it is impossible to identify a trypanosome inoculable to rats (*T. vivax*) with one which is not thus inoculable (*T. cazalboui*). They consider that *T. vivax* cannot be employed as a name for any known trypanosome. Bruce *et al.* (1910*e*), on the other hand, came to the conclusion that *T. vivax* and *T. cazalboui* are identical. They compared the Uganda strain, which Laveran

had examined and pronounced to be *T. cazalboui*, with Ziemann's original preparations, and could find no difference between them. If they are not identical, it means that Ziemann's *T. vivax*, which was discovered by him in numbers of animals over a wide area, has not been rediscovered. This is highly improbable. It seems far more likely that *T. vivax* is the active trypanosome which has been found in the blood of cattle, sheep, and goats by many observers in various parts of Africa, and which is not inoculable into laboratory animals, and that Ziemann and Cazalbou were working with the same trypanosome. The results of inoculations given by Ziemann agree with this, apart from the eight grey rats which he states became infected. In this case he may have been using a specially virulent strain; or, as seems more probable, he may have been dealing with a mixed infection of two trypanosomes (*T. vivax* and a small trypanosome like *T. congolense*), one of which was inoculable to rats, as has been suggested by Yorke and Blacklock (1911) and Blacklock (1912). A similar suggestion was made by Kleine and Fischer (1912), and it seems probable that though Ziemann recognized the typical very active form (*T. vivax*), in some of his inoculations he injected it along with another one (*T. brucei*) which he did not recognize, and which infected his grey rats. It seems hardly justifiable to abandon Ziemann's name, *T. vivax*, for this form because of the single discrepancy when it conforms in other respects with the trypanosome which has been studied subsequently, and probably with greater accuracy. There is another point which must be mentioned. Ziemann gave as the dimensions of his trypanosome a length of 18 to 26 microns, with some forms reaching 30 microns. Laveran and Mesnil (1912) give for *T. cazalboui* an average length of 21 microns. Therefore, Ziemann's measurements are higher than the latter's. Bruce's measurements for *T. vivax*, however, agree with Ziemann's, as do those of Rodhain, Pons, Vandenbranden, and Bequært (1913*a*) for a trypanosome of the *T. cazalboui* type seen by them in the Belgian Congo; and, as noted above, Laveran examined Bruce's Uganda strain, and pronounced it to be *T. cazalboui.*

Taking all these facts into consideration, there can be little doubt that the trypanosome generally called *T. cazalboui* by French workers is the same as the one seen by Ziemann and named *T. vivax*, a name which, on account of the trypanosome's motility, is a particularly suitable one. The trypanosome which Kleine (1910) named *T. bovis*, and which he discovered in cattle in the Tanganyika district, is almost certainly *T. vivax*, as also that referred to as *T. angolense* by Broden and Rodhain (1910) in the Congo. Walravens (1924) has given the name *T. rodhaini* to a trypanosome found in the pig in the Belgian Congo. It resembles *T. vivax* in having a flagellum, but differs in being much less active and in having a narrow body. As no measurements are given, it is evident further investigations are required before the validity of the species can be accepted.

Distribution.—*T. vivax* is widely distributed throughout the tsetse fly areas of Africa. It has been found most commonly in cattle, sheep, and goats, but also occurs in equines. The infected animals usually die in from fifty to ninety days. According to Hornby (1921), *T. vivax* is generally less virulent to cattle than *T. congolense*, and a certain number of the animals recover naturally. They are, however, easily reinfected. Goats may recover from their infection, but the other animals rarely do. As remarked above, monkeys, dogs, guinea-pigs, rats, and mice are not inoculable.

Blacklock and Yorke (1913*a*) have shown, however, that rabbits may

sometimes be inoculated and the strain carried on in them. Kleine (1923) states that on one occasion he produced a transitory infection in a monkey.

Morphology.—*T. vivax* can be distinguished from other pathogenic trypanosomes, not only by its activity, which enables it to dash about in a fresh blood preparation with great energy, but also by its morphological features (Fig. 232, B, and Plate V., F, p. 456). It measures 18 to 26 microns and has a definite flagellum. As regards the structure of the body, the bulk of the cytoplasm lies posterior to the nucleus, giving to this part of the body, which consists of a clear alveolar cytoplasm, a swollen and broad appearance. The body narrows at the nucleus and tapers off to the anterior end. The kinetoplast is at or near the posterior extremity, and is well developed. The nucleus is central, while the undulating membrane is less developed and the axoneme straighter than in *T. brucei* or *T. evansi*. The flagellum is 3 to 6 microns in length.

Transmission and Reservoir.—*T. vivax* was found in the blood of a bush buck by Bruce *et al.* (1910*e*) in Uganda. Rodhain, Pons, Vandenbranden, and Bequært (1912) recovered the trypanosomes from various antelopes in the Belgian Congo, as also did Kinghorn and Yorke (1912*a*) in North-East Rhodesia (see p. 508).

Several species of tsetse fly are capable of transmitting *T. vivax*. Bruce *et al.* (1910*a*, 1911*h*) found that development took place in about 20 per cent. of *Glossina palpalis* fed on infected animals. These flies were also found naturally infected. The researches of Pecaud (1909), Bouffard (1909, 1910), Bouet and Roubaud (1910, 1911*a*), and Roubaud (1910) have shown that the trypanosome with which they worked, and which they called *T. cazalboui*, could be transmitted by *G. palpalis*, *G. tachinoides*, *G. longipalpis*, and by *G. morsitans*, while Rodhain, Pons, Vandenbranden, and Bequært (1912) also transmitted it by *G. morsitans*.

Cycle in the Fly.—The development in the fly as first noted by Bruce *et al.* (1910*a*) illustrates a third type of evolution (Fig. 219). In this case there is no stomach phase of development, the multiplication of the trypanosomes taking place in the proboscis only. Crithidia forms are produced in the labial cavity, and these attach themselves in large numbers to its walls. The hypopharynx is invaded, and finally there are produced the infective metacyclic trypanosomes of the blood type.

Lloyd and Johnson (1924) find that the trypanosomes taken into the gut quickly degenerate, and can thus be distinguished from *T. gambiense*, *T. brucei*, and *T. congolense*, which develop in this situation (Fig. 233). In the labial cavity they quickly change into crithidia forms with flagella and become attached to the walls in compact colonies. When a colony is small the flagellates are short and boat-shaped, and when it is large

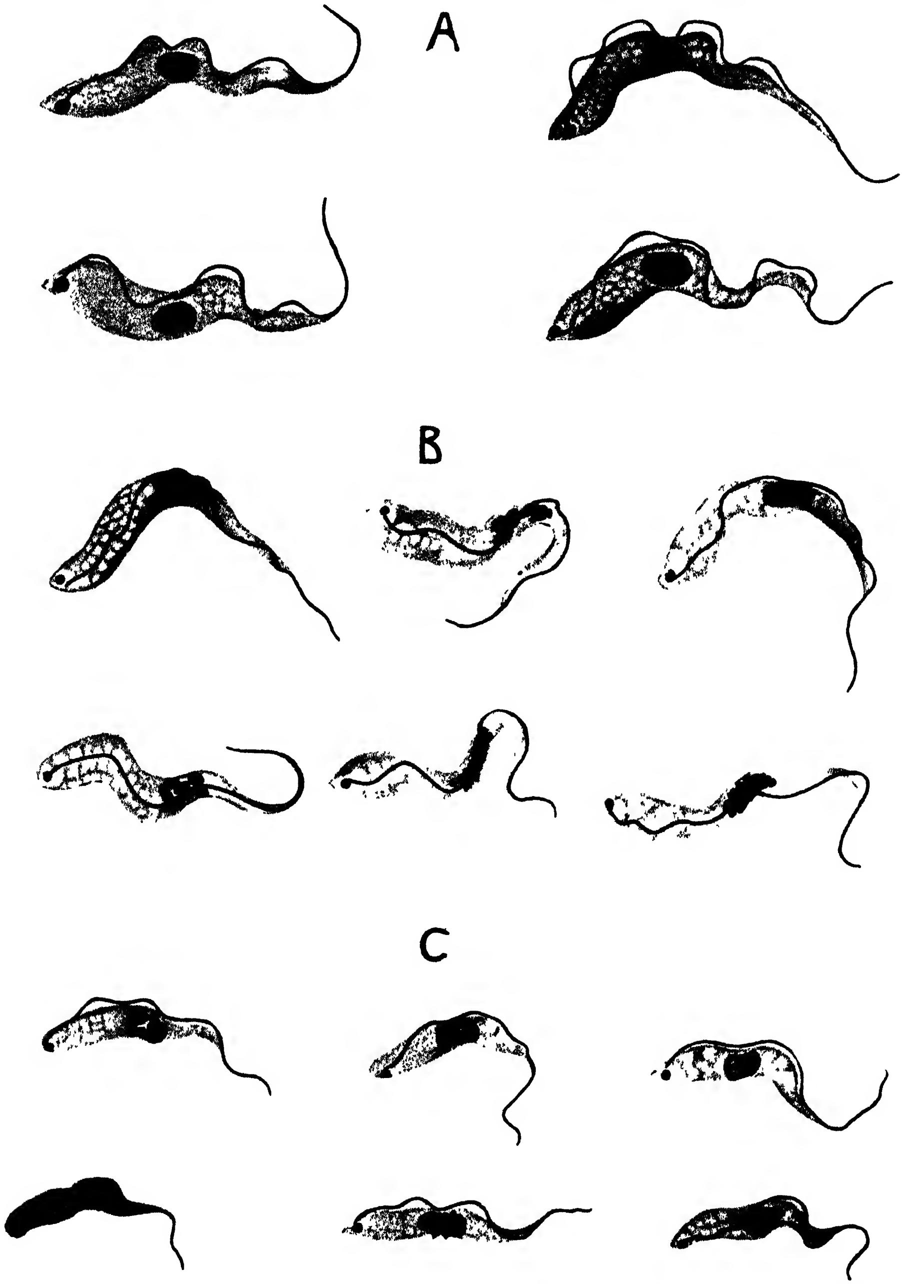


FIG. 232.—TRYPANOSOMES OF THE *T. vivax* GROUP (×2,000). (AFTER BRUCE, HARVEY, HAMERTON, MACKIE, AND LADY BRUCE, 1911 AND 1913.)

A. *Trypanosoma capræ*. B. *Trypanosoma vivax*. C. *Trypanosoma uniforme*.

they are long and scroll-like. The posterior end is seldom truncated, as in the corresponding forms of *T. congolense*. At a later stage the nucleus and kinetoplast are close together at the posterior end of the body, and it is these forms which invade the hypopharynx. The nucleus then moves forward, and the infective metacyclic trypanosomes are produced. These are slender, markedly undulant trypanosomes with sharply-pointed

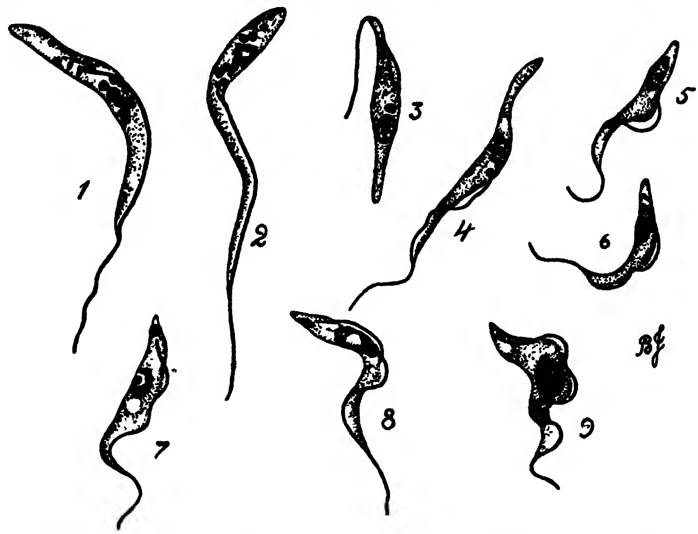


FIG. 233.—DEVELOPMENTAL FORM OF *T. vivax* IN *Glossina tachinoides* (×2,000). (AFTER LLOYD AND JOHNSON, 1924.)

1-2. Degenerate forms in mid-gut and crop.
3-4. Crithidia forms from fixed colonies in labial cavity.
5-6. Pre-infective forms in labial cavity.
7-9. Infective forms in hypopharynx (*G. morsitans* and *G. palpalis*).

posterior ends and a free flagellum, which is from one-third to one-fourth the length of the body. Measurements of a number of infective forms from three species of tsetse fly gave the following average dimensions:

	Length.	*Length of the Flagellum.*	*Breadth at Nucleus.*
Glossina tachinoides	14·4	3·9	1·8
Glossina palpalis	14·9	4·3	1·9
Glossina morsitans	14·8	4·1	1·6

By means of the above characters it is possible to recognize a *T. vivax* infection in tsetse flies without the necessity of infecting animals (see p. 515).

Mechanical transmission by means of *Stomoxys* was effected by Bouffard (1907) and by Bouet and Roubaud (1912*a*).

Possibility of T. vivax Infecting Man.—An observation of Macfie (1917*b*) is of considerable interest in connection with this trypanosome. He discovered in two blood-films made on two occasions from a native of the Gold Coast a trypanosome which morphologically resembled *T. vivax*. Of 200 trypanosomes measured, the longest was 24 microns and the shortest 18 microns, giving an average of 20·7 microns. The organism was evidently monomorphic, and completely unlike the ordinary *T. gambiense* of this district. Furthermore, it had the swollen and rounded posterior end of *T. vivax*, and large terminal or nearly terminal kinetoplast. As *T. vivax* is exceedingly common in domestic animals in West Africa, the author, having demonstrated its presence in 76 per cent. of the hump-backed cattle of Accra, it is possible that in this case *T. vivax*, usually not inoculable to man, has been able to obtain a footing in a human host. Macfie is inclined to regard the infection as actually one of *T. vivax* in man. Should this conclusion be confirmed, it is of interest in the light of the much-disputed relationship of *T. brucei* and *T. rhodesiense*, where again a trypanosome which is readily inoculable to domestic animals may only infect human beings under exceptional circumstances. It, furthermore, raises the question of the possibility of other trypanosomes infecting human beings.

Possibility of T. vivax occurring in South America.—Leger, M. and Vienne (1919) discovered a trypanosome in cattle in Venezuela, which they named *T. guyanense*. As this name was already pre-occupied (Mesnil, 1912), Lavier (1921) proposed to substitute the name *T. viennei*. As regards its morphological characters and the susceptibility of laboratory animals, it resembled *T. vivax* of Africa. Tejera (1920*a*) studied the organism, and thought it possible that it was actually *T. vivax* which had been introduced from Africa some years before. If this view is correct, it is remarkable that the trypanosome should have established itself in South America, where the tsetse fly, the natural vector of *T. vivax*, does not occur.

Trypanosoma capræ Kleine, 1910.—This trypanosome was first studied by Kleine (1910) near Tanganyika, and was afterwards investigated by Bruce *et al.* (1913*f*) in Nyasaland. It is a very actively motile trypanosome, like *T. vivax*, which it resembles closely (Fig. 232, A, and Plate V., E, p. 456). It is, however, more heavily built, has a larger and more clumsy appearance, and varies in length from 18 to 32 microns, with an average of 25·5 microns. Measured across its broadest part, which, as in *T. vivax*, is posterior to the nucleus, it is found to vary in breadth from 1·75 to 4·35 microns, with an average of 3 microns. The undulating membrane is broader than in *T. vivax*, and there is a flagellum 4 to 9·5 (average 6·5) microns in length. It occurs in cattle, sheep, and goats, which may recover from their infection. It is not inoculable to small animals in the laboratory.

As demonstrated by Fehlandt (1911), and by Bruce *et al.* (1913*f*, 1914*e*), *T. capræ* is transmitted by *Glossina morsitans.* Bruce *et al.* (1914*b*) also effected transmission by means of *G. brevipalpis.* There is no stomach phase of development in the fly, the whole cycle taking place in the labial cavity and hypopharynx. Bruce and his co-workers (1913) found that 11·1 per cent. of the wild game harboured this trypanosome.

Trypanosoma uniforme Bruce *et al.*, 1911.—This trypanosome was first studied and named by Bruce *et al.* (1911*a*) in Uganda. It is a small form of the *T. vivax* type (Fig. 232, C, and Plate V., D, p. 456). Its movements, though vigorous, cannot be compared with those of *T. vivax.* The anterior part of the body does not show the same degree of narrowing as in *T. vivax,* so that there is not so great a contrast between the width of the body anterior and posterior to the nucleus. The post-nuclear region of the body, however, is decidedly bulbous and the posterior end rounded. *T. uniforme* varies in length from 12 to 19 microns, with an average of 16 microns. The width is from 1·5 to 2·5 microns. The kinetoplast is well developed and near the posterior extremity. The membrane is distinct, though narrow, and there is a flagellum 2 to 5 microns in length.

Like *T. vivax* and *T. capræ,* this trypanosome affects cattle, sheep, and goats, but is not inoculable to the smaller animals. The animals infected usually die in about thirty to sixty days.

Glossina palpalis was shown by Fraser and Duke (1912*c*) in Uganda to be the carrier of *T. uniforme.* The development is confined to the proboscis, as in *T. vivax* and *T. capræ.* Flies do not become infective till twenty-seven to thirty-seven days after feeding. It was also shown that the trypanosome was harboured by antelope on the lake shore in Uganda. It was the only trypanosome isolated from wild animals, including thirty-two lake-shore antelope, though the *G. palpalis* of the area examined were known to be infected with *T. gambiense* and *T. vivax.* A healthy goat was fed upon by 1,020 flies collected on the lake shore. The animal first showed *T. uniforme* in its blood, and some days later *T. vivax* also.

It will be noted that the three trypanosomes, *T. vivax, T. capræ,* and *T. uniforme* resemble one another very closely. They differ only in their average dimensions. It is open to question whether they represent distinct species or should be regarded as merely varieties or races of *T. vivax.*

2. PATHOGENIC TRYPANOSOMES TRANSMITTED BY SPECIES OF TABANUS OR OTHER BLOOD-SUCKING ARTHROPODA. MONOMORPHIC TRYPANOSOMES PROVIDED WITH FLAGELLA.

The trypanosomes included in this group are placed provisionally amongst the forms which develop in the anterior station in the invertebrate. In no case, however, have the details of the development been worked out.

As far as present information goes, it appears that the trypanosomes are transmitted by biting flies in a purely mechanical manner, but it is possible that a definite developmental cycle will be discovered. Should the method of transmission prove to be purely mechanical, then a new group would have to be formed to include them.

Trypanosoma evansi (Steel, 1885).—Synonyms: *Spirochæta evansi* Steel, 1885; *Trichomonas evansi* (Crookshank, 1886); *Herpetomonas evansi* (Crookshank, 1886); *Trypanosoma evansi* var. *mborii* Laveran, 1903; *T. berberum* Ed. and Et. Sergent, 1904; *Trypanozoon evansi* (Lühe, 1906); *Trypanosoma soudanense* Laveran, 1907; *T. hippicum* Darling, 1910; *T. venezuelense* Mesnil, 1910; *T. annamense* Laveran, 1911; *T. marocanum* Sergent, Lhéritier and Belleval, 1915; *Castellanella evansi* (Chalmers, 1918); *T. equinum* Voges, 1911 (?).

Under the name of surra, a disease of horses had been known for many years in India. Evans (1880) described an organism he found in the blood of horses, camels, and mules suffering from this disease. It was rediscovered by Steel (1885), who regarded it as a spirochæte, but the work of Crookshank (1886) and others revealed its true nature. The disease as it occurs in India was the subject of lengthy reports by Lingard (1893). Surra is now known to be caused by *T. evansi*, which is found naturally in horses, mules, donkeys, cattle, camels, elephants, and dogs. It is, furthermore, inoculable into most of the laboratory animals.

Distribution. — Owing to the movement of horses about the world, surra is now a widespread disease. It occurs in India, Burma, Assam, Ceylon, South China, Siam, Sumatra, Java, Philippines, Mauritius, Madagascar. Animals afterwards found to be infected have been imported to Australia and the United States, but precautionary measures have prevented any extension of the disease. From India it extends into Persia, South Russia, Mesopotamia, and Arabia.

Susceptibility of Animals. — The disease in horses is nearly always fatal in a period varying from a week to six months. The infected animals show fever, loss of appetite, anæmia, wasting, and various œdemas. Similar symptoms are to be noted in camels, in which, however, the duration may extend to three years, while spontaneous recovery may take place. As a rule, the disease in cattle is of a milder type. *T. evansi* appears to be much less virulent to cattle than to horses. The animals show few symptoms as a rule, and nearly always recover naturally, but outbreaks affecting cattle seriously have been described from Java by Penning (1899, 1900) and Schat (1902), and in Mauritius by Edington and Coutts (1907). Elephants are affected very much as camels are. An observation by Cameron, recorded by Evans (1910) and Evans and Rennie (1910), is of interest in this connection. Trypanosomiasis was discovered in a herd of seven to nine elephants at Pyinmana in Burma. The trypanosome

morphologically and in inoculations appeared to be *T. evansi*. The animals were in poor condition and suffered from fever. Treatment with liquor arsenicalis was carried out over a long period, during which the animals were kept at work, and in two to three years they not only recovered clinically, but their blood ceased to be infective to rats. Dogs are very susceptible to *T. evansi*, and in India hunting packs have sometimes suffered heavily. Death may occur in a week, or not till three or four months after infection. Cats can be infected, as also pigs. In experimental inoculations rats and mice develop very large infections, and die in about a fortnight. In guinea-pigs the infection is not so intense, and death results in about one month. Rabbits show still milder infections, but the animals die in about the same period. Monkeys are also susceptible, and the disease produced terminates fatally in about two months. According to Laveran (1904*a*), baboons (*Cynocephalus*) are immune. Sheep and goats, though they sometimes contract a fatal infection, generally recover after six months. During this period the trypanosomes may be so scanty in the blood that they can only be demonstrated by inoculation of blood to more susceptible rats or guinea-pigs. Goats which have recovered from their infection are found to be immune to reinoculation. Laveran and Mesnil have employed these immune animals in the differentiation of *T. evansi* from other nearly allied forms.

The virulence of a strain of *T. evansi* is greatly increased by successive passages through small animals. In the first few passages after inoculation from an infected horse the duration of life in these animals is at least double what it will be later in sub-inoculations.

Morphology.—*T. evansi* is a monomorphic trypanosome which always possesses a flagellum (Fig. 234 and Plate V., C, p. 456), though Bruce (1911) states that rarely short stumpy forms without flagella occur. In this respect it differs from *T. brucei* and *T. gambiense*, which are definitely polymorphic, in that the short stumpy forms are frequently found. Measuring 820 individuals, Bruce (1911) found a variation in length of *T. evansi* between 18 and 34 microns, with an average of 24·9. The breadth is given as varying between 1·5 and 2 microns. The curve (Fig. 196) shows the percentage of trypanosomes of various lengths from a large number measured as compared with *T. brucei*.

Transmission.—Surra is transmitted from animal to animal by various blood-sucking flies, chiefly those belonging to the genus *Tabanus* (Fig. 211). Up to the present no evidence of a cycle of development comparable with that of *T. gambiense* and other trypanosomes in tsetse flies has been demonstrated for *T. evansi*. Rogers (1901) in India recorded successful transmission experiments. "Horse flies" were allowed to feed partially on infected dogs, and then to complete their meal on healthy dogs,

some of which became infected. If the interval between the feeds was over twenty-four hours, no infection took place. Musgrave and Clegg (1903) in the Philippines transmitted surra by biting flies. Monkeys, horses, dogs, rats, and guinea-pigs were thus infected. In one experiment the house fly carried the infection from an infected to a healthy dog by feeding successively upon a wound on each. In a similar manner fleas were shown to be capable of carrying infection between dogs and cats. Working with a North African strain (*T. berberum*) Sergent, Ed. and Et. (1905*b*, 1906*a*), effected a mechanical transmission with *Stomoxys* and

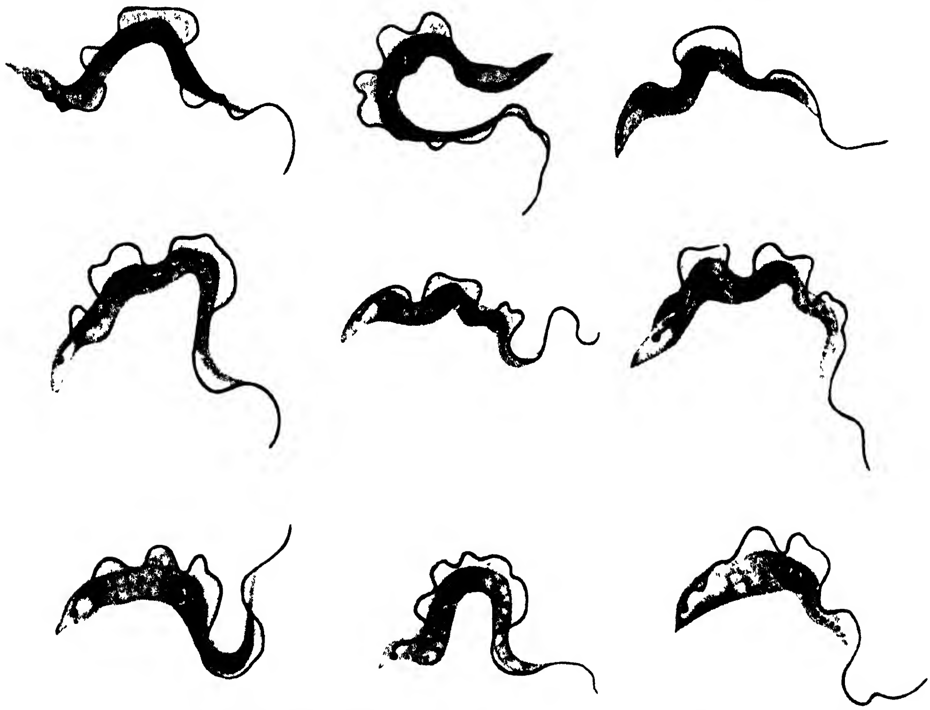


FIG. 234.—*Trypanosoma evansi* FROM BLOOD OF VARIOUS ANIMALS (×2,000). (AFTER BRUCE, 1911.)

Tabanus nemoralis. Fraser and Symonds (1908), working in the Federated Malay States, found that four species of *Tabanus* (*T. fumifer*, *T. partitus*, *T. vagus*, and *T. minimus*) would convey the trypanosome if not more than five minutes elapsed between the feeds on the infected and healthy animals. With species of *Stomoxys* and *Hœmatopota* they obtained negative results. Leese (1909) at Mohand in U.P., India, obtained positive results with *Tabanus*, *Hœmatopota*, and *Stomoxys*, and he records an outbreak of the disease among horses where the only biting fly was *Lyperosia minuta.* Baldry (1911) at Muktesar in India inoculated the

intestinal contents of various species of *Tabanus* (*T. orientis*, *T. tropicus*, *T. subcallosus*) and *Stomoxys calcitrans* into guinea-pigs at varying intervals after feeding on infected horses. Up to twenty-four hours the animals became infected, but not later. Bouet and Roubaud (1912*a*), working with a Sudan strain (*T. soudanense*), effected transmission with *S. calcitrans*. Mitzmain (1913) conducted very careful experiments in the Philippines with *T. striatus*, which were bred in the laboratory. In these experiments the trypanosome was transmitted by the method of interrupted feeding, where only a short interval intervened between the two feeds. It was further shown that the contaminated labellum did not appear to be a factor in the conveyance of the trypanosomes, which were present in the gut of the fly up to thirty hours after feeding. Transmission was also effected with *S. calcitrans*, and in one instance by means of the louse, *Hæmatopinus tuberculatus*. Sergent and Donatien (1922), working with the strain known as *T. berberum*, again obtained a mechanical transmission with *Stomoxys*, while Donatien and Lestoquard (1923) observed that dogs which frequented the stables occupied by infected dromedaries became infected through the numerous *Stomoxys* which were always present. It will thus be seen that up to the present the only known method of transmission of *Trypanosoma evansi* in nature is a mechanical one, in which various biting insects inoculate healthy animals within a short time of their having fed on infected ones. It would seem very probable, however, that this is not the whole of the story, and that further research will reveal some form of development in the fly, leading to a permanent infection similar to that which occurs in various species of *Glossina* in Africa.

Cross and Patel (1921) in India claim to have transmitted *T. evansi* from camels to healthy rabbits by means of ticks. A number of ticks (*Ornithodorus crossi* and *O. laborensis*) were fed on camels. Some were allowed to complete their feed, while others were interrupted before this was finished. Those which had not completed their feed were allowed to finish it upon healthy animals from one to twenty minutes later. The others were similarly fed again five to twenty-two days later on healthy animals. In no case did infection result. After forty-six days the result was again negative, but after sixty-seven days forty-two of the ticks, together with two others which had fed on an infected camel twenty-two days before, produced an infection in a healthy rabbit. After a further interval of sixteen days thirty-six ticks were fed on a clean rabbit, and again after eighteen days on another rabbit. Both these animals became infected. It is concluded that ticks can harbour the virus for long periods (67 to 101 days), and then produce outbreaks of surra. Trypanosomes first appeared in the rabbits eight to ten days after the ticks had fed,

but no statement is made as to whether the rabbits died of their infections or not. In a further series of experiments, Cross (1923) confirms his original findings. The ticks transmitted the trypanosome seventeen days and one month after feeding on an infected animal. He thinks it probable that a cyclic development occurs in the ticks. He has also transmitted the trypanosome mechanically by means of *Tabanus albimedius*, when the feeds on the infected and uninfected animals followed one another immediately. Yorke and Macfie (1924) report that they received about 200 *O. crossi* from Cross in India. Though the ticks had been fed on an infected dog in India, and after their arrival in Liverpool were found to contain well preserved, though motionless, trypanosomes, they failed to infect rabbits on which they were fed. The writer also failed to infect rabbits and rats with a batch of similar ticks received from Cross. Though Singh (1925) states that he has confirmed the observations of Cross and Patel, the subject is one which requires further investigation.

Reservoir.—The question of a reservoir host for *Trypanosoma evansi* has been frequently raised. Camels, in which the disease pursues a chronic course, must act in this way, as also the buffalo, which may carry the trypanosome without suffering to any great extent. Baldry (1910) expressed the opinion that the pig was a source of infection for other animals, and he showed that it was susceptible to inoculation.

Treatment.—As regards treatment, the best results have been obtained by the use of atoxyl subcutaneously and arsenious acid by the mouth, as recommended by Holmes (1910) in India. Maya (1912) had good results with this treatment in Mauritius. Thiroux and Teppaz (1910) report favourably on the action of orpiment by the mouth associated with atoxyl or tartar emetic injections, while Cross (1920, 1920*a*) found that tartar emetic intravenously gave promise of success.

Other Trypanosomes of the Trypanosoma evansi Type.

Forms in Asia and Africa.

Trypanosoma evansi var. **mborii** Laveran, 1911.—A disease of dromedaries known as mbori was first described by Cazalbou (1903) in the French Sudan. It occurs in the districts of the Niger and Senegal Rivers, and was first noted by its discoverer at Timbuctoo. It affects horses as well as dromedaries, and produces a disease similar to surra. Laveran (1904*c*) considered the trypanosome which causes the disease to be a variety of *T. evansi*, and he (1911) named it *T. evansi* var. *mborii*. It is inoculable into the same animals as *T. evansi*, but is less virulent. Morphologically it is indistinguishable from the trypanosome of surra.

A trypanosome of the same type has been recorded as producing a

disease in dromedaries in Italian Somaliland by Martoglio (1911), and in South-West Africa by Reinecke (1911). Theiler (1906*b*) met with the same trypanosome in South Africa in dromedaries which had come from Somaliland.

T. annamense Laveran, 1911.—Another trypanosome morphologically indistinguishable from *T. evansi* is one first noted by Blanchard (1888) in horses in Tonkin and Annam. It has been studied by various observers, and found to occur also in cattle. Laveran (1911) studied the trypanosome, and found that goats which had acquired an immunity to the true *T. evansi* of India could still be infected with the Annam strain. Accordingly, he designated the trypanosome *T. annamense.*

T. soudanense Laveran, 1907.—Another disease caused by a trypanosome, and again in the same animals, is the debab of Algeria and Egypt, and probably North Africa generally. It extends into the same districts in which Cazalbou studied the disease mbori. A strain of this trypanosome, which was isolated from a camel, was studied by Laveran (1907) by immunity tests in goats. This led him to regard it as a species distinct from that causing mbori. The trypanosome, which he named *T. soudanense,* is not distinguishable from *T. evansi* save by its immunity reactions. It is possibly this trypanosome or the variety of *T. evansi* causing mbori which is responsible for the disease of camels in Khordofan and Somaliland.

T. berberum Sergent, Ed. and Et., 1904, and **T. marocanum** Sergent, Lhéritier, and Belleval, 1915—These two trypanosomes of the *T. evansi* type are also recorded from North Africa. *T. berberum* produces a disease of camels and horses similar to debab throughout North Africa, while *T. marocanum* was encountered in an outbreak amongst horses at Casablanca. On the evidence of cross-immunity tests these trypanosomes were stated to differ from one another and also from *T. evansi.* Sergent, Ed. and Et., and Donatien (1920) have shown that *T. berberum* may, at the height of an infection, pass through the placenta and bring about infection and death of the young *in utero.* Camels which have passed the acute stage of the disease bear healthy young, which, however, possess no immunity to infection. Vialatte (1915) and Donatien and Parrot (1922) have reported *T. berberum* as occurring naturally in dogs, while similar observations for *T. marocanum* have been made by Delanoë (1920) and Velu (1920).

A trypanosome of camels in Russian Turkestan was named *T. ninæ kohl-yakimov* by Yakimoff (1921*a*), who claims that it differs from *T. evansi* in pathogenicity to laboratory animals and serum reactions, tests which are quite insufficient to justify the creation of a new species.

It will be seen from the above account that these various supposed species produce diseases in those animals which are known to suffer from surra. Moreover, they are morphologically indistinguishable from

T. evansi, from which they have been separated by Laveran and others by cross-immunity tests alone. They resemble *T. evansi* in that tabanid flies are probably responsible for their transmission. It is therefore a reasonable hypothesis to suppose that they are merely races of *T. evansi*. The results of inoculation and immunity tests are merely indications of a difference in virulence of various strains of the same trypanosome.

Forms in Central and South America.

In America, domestic stock is also liable to infection with trypanosomes of the *T. evansi* type, and it seems probable that these also may be merely races of *T. evansi*.

Trypanosoma hippicum Darling, 1910.—This trypanosome, which very closely resembles *T. evansi*, was first seen by Darling (1910) in mules arriving in Panama from the United States. It produces in equines a disease which is very like surra. It is inoculable into laboratory animals, in which it gives rise to the same types of infection as those caused by *T. evansi*. Laveran and Mesnil (1912) state that the large forms sometimes seen in *T. evansi* infections do not occur in the case of *T. hippicum*, and that it can be distinguished from the trypanosome of surra by cross-immunity tests.

T. venezuelense Mesnil, 1910.—This form, which was first seen by Rangel (1905), is very similar to *T. hippicum* and *T. evansi*, and causes a disease of equines and dogs in Venezuela.

Morphologically it is indistinguishable from either, and as no cross-immunity tests had been carried out at that time, Mesnil (1910), who examined a strain sent to Paris, considered it safer to give it a new name provisionally. Leger and Tejera (1920) have recently investigated this trypanosome, and compared it with *T. evansi*. They claim that it differs from *T. evansi* in dimensions, in virulence for laboratory animals, and response to various medicaments and blood-sera. Taking these facts into consideration, together with the results of cross-immunity tests, they conclude that *T. venezuelense* is a distinct species. The comparisons were made, however, with a strain of *T. evansi* which had long been maintained in laboratory animals. It is very questionable if the slight differences noted justify the retention of *T. venezuelense* as a distinct species. Rangel (1905) stated that the trypanosome occurs naturally in the domestic dog, the wild dog (*Canis azare*), capibara (*Hydrochœrus capibara*), and howler monkeys (*Mycetes ursinus* and *M. seniculus*).

T. equinum Voges, 1901.—Synonyms: *T. equina* Voges, 1901; *T. elmassiani* Lignières, 1902; *Trypanozoon equinum* (Lühe, 1906).

This is a trypanosome which produces a disease of horses known as mal de Caderas. It occurs in various parts of South America (Brazil,

Bolivia, Paraguay, Argentine). Mules and donkeys also acquire the disease, but in them it is less acute than in horses. Cattle, sheep, and goats, which usually recover, take the disease in a very mild form, trypanosomes only being demonstrable by inoculation of the more susceptible smaller animals. The duration of the disease in horses varies from about one to four or five months. Voges (1901) quotes an instance in which a regiment received 600 horses, of which 500 died of the disease in the course of the succeeding five months. Inoculated to the smaller laboratory animals, an acute infection is produced comparable with those produced by *T. evansi* and *T. brucei.*

T. equinum is remarkable in that it differs from all known pathogenic trypanosomes in the absence of the kinetoplast, or rather the parabasal body, for the axoneme can still be seen to originate in a minute blepharoplast, as is well illustrated in the figures of detached flagella depicted by Sivori and Lecler (1902). In length it measures from 22 to 24 microns, of which about five comprise the flagellum (Plate V., I, p. 456). Dividing forms may be as much as 30 microns in length. The breadth of the trypanosome is 3 to 4 microns. The nucleus is central, and there is a well-developed membrane. *T. equinum*, apart from the condition of the kinetoplast, of which the blepharoplast alone is present, closely resembles *T. evansi.*

It has been noted that from time to time epidemics occur amongst the capibaras (*Hydrochœrus capibara*) in districts in which *T. equinum* is endemic. Migone (1910) studied one of these outbreaks, found trypanosomes resembling *T. equinum* in the blood, and noted that the animals died with symptoms which he stated resembled those of mal de Caderas. The evidence, though not absolutely conclusive, seems to suggest that these animals may act as a reservoir for the virus, though the fact that they die of the infection does not support this view. The disease is probably transmitted by species of *Tabanus* and *Stomoxys.* Sivori and Lecler (1902) claimed to have obtained mechanical transmission by means of *S. calcitrans.*

These various South American trypanosomes resemble *T. evansi* so closely that it seems more reasonable to regard them as races of *T. evansi* rather than distinct species. In connection with the absence of the parabasal body in *T. equinum*, it must be remembered that similar forms in other trypanosomes can be produced experimentally by the action of certain drugs (p. 460).

II. PATHOGENIC TRYPANOSOMES PASSED DIRECTLY FROM VERTEBRATES TO VERTEBRATES.

Trypanosoma equiperdum Doflein, 1901.—Synonyms: *T. rougeti* Laveran and Mesnil, 1901; *Trypanozoon equiperdum* (Lühe, 1906); *Castellanella equiperdum* (Chalmers, 1918).

Unlike other pathogenic trypanosomes, *T. equiperdum* is transmitted directly from animal to animal during the sexual act, as occurs with the organism of syphilis. It produces in horses and donkeys a disease known as dourine, which is endemic in various countries of Europe, in India and probably other parts of Asia, in North Africa, North and South America, and Canada. It was first named *T. equiperdum* by Doflein (1901), and a few days later *T. rougeti* by Laveran and Mesnil.

Symptomology.—The disease is usually of a chronic nature. The first symptoms are noted about ten days or a fortnight after infection, and consist of œdema of the sexual organs. About a month later characteristic lesions in the shape of plaques appear on the skin. These vary in size from that of a shilling to the palm of the hand. They are raised, and give the impression of a hard subcutaneous disc. Each plaque may persist for several days, or it may disappear in a few hours. A period of gradual weakening and loss of flesh supervenes, accompanied by fever and progressive anæmia. Finally, paraplegia and various nervous symptoms appear, and the animal dies in from two months to a year after infection. The females usually abort during the course of the disease. Very rarely recovery has taken place, after which the animals are immune to reinfection. Sergent, Donatien, and Lhéritier (1920) have shown that horses which have entirely recovered as judged by disappearance of clinical symptoms, either naturally or as a result of treatment, may still transmit the disease.

Stallions which had acquired the disease were treated with atoxyl and orpiment till complete clinical recovery had taken place. The animals were then returned to full regimental duty, but the blood was examined from time to time by inoculating dogs. The following history of four stallions is given:

1. For a month after complete clinical recovery the blood still infected dogs. During three years 3½ litres of blood injected into nineteen dogs failed to infect any.

2. For a year after recovery 1½ litres of blood failed to infect eight dogs. Four months later one of two dogs injected became infected. During the next two years 2 litres did not infect any of ten dogs.

3. During two years 2·2 litres of blood did not infect eleven dogs. During the third year, however, dogs were infected.

4. During three and a quarter years 3·64 litres of blood did not infect nineteen dogs. The blood then infected one of two dogs injected.

Watson (1920) studied an infected mare, which suffered from three to four day periods of fever every twenty-four to twenty-eight days associated with œdematous swellings of similar duration. These swellings were examined every few hours by abstraction of serum with a fine needle, and the trypanosomes were found to pass through a definite cycle. The first specimens of serum showed few organisms. Later they increase in number till at the fortieth hour agglomerations were present. At about the forty-fourth hour all the trypanosomes were found to have been ingested by the macrophages. At forty-eight hours only débris of trypanosomes could be recognized in the cells, while on the third day no trace of them could be found and the swelling disappeared.

Watson found that the virulence of *T. equiperdum* for horses was increased after passage through the mouse, and the infection produced was associated with the constant presence of trypanosomes in the blood-stream, a condition never observed in the natural disease or in horses experimentally infected by injection of trypanosomes directly from a naturally occurring case of dourine.

The discovery of the trypanosome in the naturally infected horses and donkeys is often very difficult. It occurs in very small numbers in the blood-stream, but is more numerous in the exudate from the areas of œdema and in fluid obtained from the plaques. Watson (1920) believes that the organism is not a blood-parasite at all, and that it only occasionally gains access to the blood-stream from the connective tissue lymph channels, which constitute its usual habitat. For diagnostic purposes it is often necessary to inoculate large quantities of blood (100 to 400 c.c.) intraperitoneally to dogs. If the dogs do not become infected, this does not exclude infection in the horse. The complement fixation test, as carried out by Woods and Morris and Watson, has been referred to above (p. 452).

Susceptibility of Animals.—The trypanosome is inoculable into the dog and rabbit, and more rarely to rats, mice, guinea-pigs, monkeys, sheep, and goats. There is, however, a great variation in virulence, so that with certain strains animals are easily infected, while with others no infection takes place. Any individual strain is liable to change its virulence, so that a marked irregularity in the results of inoculations occurs. The dog seems to be the most susceptible animal, and is usually employed for purposes of diagnosis when trypanosomes cannot be found by direct examination of the blood of the horse or donkey. Dogs and rabbits infected by inoculation are able to transmit the infection during the sexual act.

Dogs usually die of an infection in two to three months. In rabbits the disease is of a chronic nature, and reveals the lesions characteristic of the

infection in horses. Recovery may take place in about a year, or death occurs before this. With virulent strains rats and mice survive from one to three weeks. Guinea-pigs succumb in one to three months. Other animals which have been inoculated generally recover.

Watson (1920) states that the Canadian strain of *T. equiperdum* was transmitted to a white mouse after hundreds of unsuccessful attempts with dogs, rabbits, guinea-pigs, rats, and mice. When once established in mice, the trypanosome was readily inoculable to the other animals. Furthermore, after passing through the horse again for several successive passages, it was readily recoverable by inoculation of laboratory animals. Thus its power of infecting laboratory animals was not lost after return to the original host. In the first instance, when the first successful inoculation of a mouse occurred, this animal was the eighty-fourth of a series of rats and mice which had been inoculated during a period of four weeks with fluid rich in trypanosomes which had been collected from the plaques appearing on an infected mare. The remaining eighty-three animals did not become infected. This change in virulence after passage through an animal is perhaps comparable with Bruce's (1914) observation that *T. congolense* (*T. pecorum*) lost its virulence for laboratory rats after passage through the goat (see p. 552). The strain isolated by Watson in mice after many failures behaved in mice and in horses like *T. evansi*. Though it was actually isolated in the first place from the serum from the œdematous swellings, it is just conceivable that the trypanosome which infected the mice was not *T. equiperdum*, but *T. evansi*, a trypanosome, however, which is not known to occur in Canada. In any case, the change in character of the trypanosome after passage through mice raises the question of relationship of these two forms.

Morphology.—*T. equiperdum* is a trypanosome of the *T. evansi* type (Plate V., C, p. 456). There is always a flagellum, and the trypanosome varies in length from 25 to 28 microns. Blacklock and Yorke (1913) examined three strains of the trypanosome obtained from various European laboratories. Two of the strains correspond with *T. equiperdum*, but one was polymorphic in nature and resembled *T. brucei*. It was concluded that this form was a different type of dourine-producing trypanosome, and it was named *T. equi*. The strain was said to have originally come from Algeria, in which country dourine is known to be due to *T. equiperdum* of the normal type. In the case of a trypanosome so far removed from its original host, quite apart from the possibility of changes in morphology, accidents of interchange with other laboratory trypanosomes may have occurred, a fallacy which certainly cannot be excluded.

Transmission.—As already remarked, dourine is spread from animal to animal by the direct contact of mucous surfaces. That the disease may

be carried in other ways has been proved to occur. Thus, Schuberg and Kuhn (1911) obtained a mechanical transmission by means of *Stomoxys calcitrans*, and Sergent, Ed. and Et. (1906*a*), with a tabanid fly, *Tabanus nemoralis*.

III. TRYPANOSOMES OF BIRDS.

The trypanosomes of birds are considered here amongst the forms which develop in the anterior station in the invertebrate, though actually in no case is the vector known, much less the type of development which occurs. Mosquitoes have been suspected of being the transmitting hosts, and certain observers have noted flagellates in the salivary glands of these insects, but there is no evidence that these have been derived from bird trypanosomes. It is quite possible, when the true intermediate host is discovered, it will be found that development of the trypanosome takes place in the posterior station.

The first satisfactory account of the occurrence of trypanosomes in birds was that of Danilewsky (1888). Since then a large number of forms has been described from well over a hundred species. In the great majority of cases, little more has been done than to give an account of the trypanosomes as they appeared in a single blood-film; no detailed study has been carried out. In one or two instances, however, more extended observations have been made. Schaudinn (1904) published a remarkable account of the development of the trypanosome (*T. noctuæ*) of the little owl. He stated that an alternation of a trypanosome with an intracellular halteridium phase occurred in the life-cycle. Subsequent observations, in spite of various attempts by Woodcock (1909) and others to substantiate Schaudinn's views, have clearly demonstrated the fallacies of his conclusions.

Trypanosoma paddæ Laveran and Mesnil, 1904.—The best account of a bird trypanosome is that of Thiroux (1905), who described the infection due to *T. paddæ* Laveran and Mesnil, 1904, in the Java sparrow (*Munia oryzivora*). The trypanosome was first seen by Levaditi in birds imported to France. By means of this strain, Thiroux was able to infect other birds and to study the course of the infection. Inoculated intraperitoneally, trypanosomes appeared in the blood of the birds in twelve hours, whereas by the intramuscular or subcutaneous method the incubation periods were nine and twelve days respectively. Furthermore, there was marked irregularity in the results of inoculation. Some birds only became infected after a second inoculation. After their appearance in the blood, the trypanosomes increased in number during nine to fifteen days, after which the number declined day by day till they could only be found with difficulty. In some cases the infection brought about the death of the birds. The trypano-

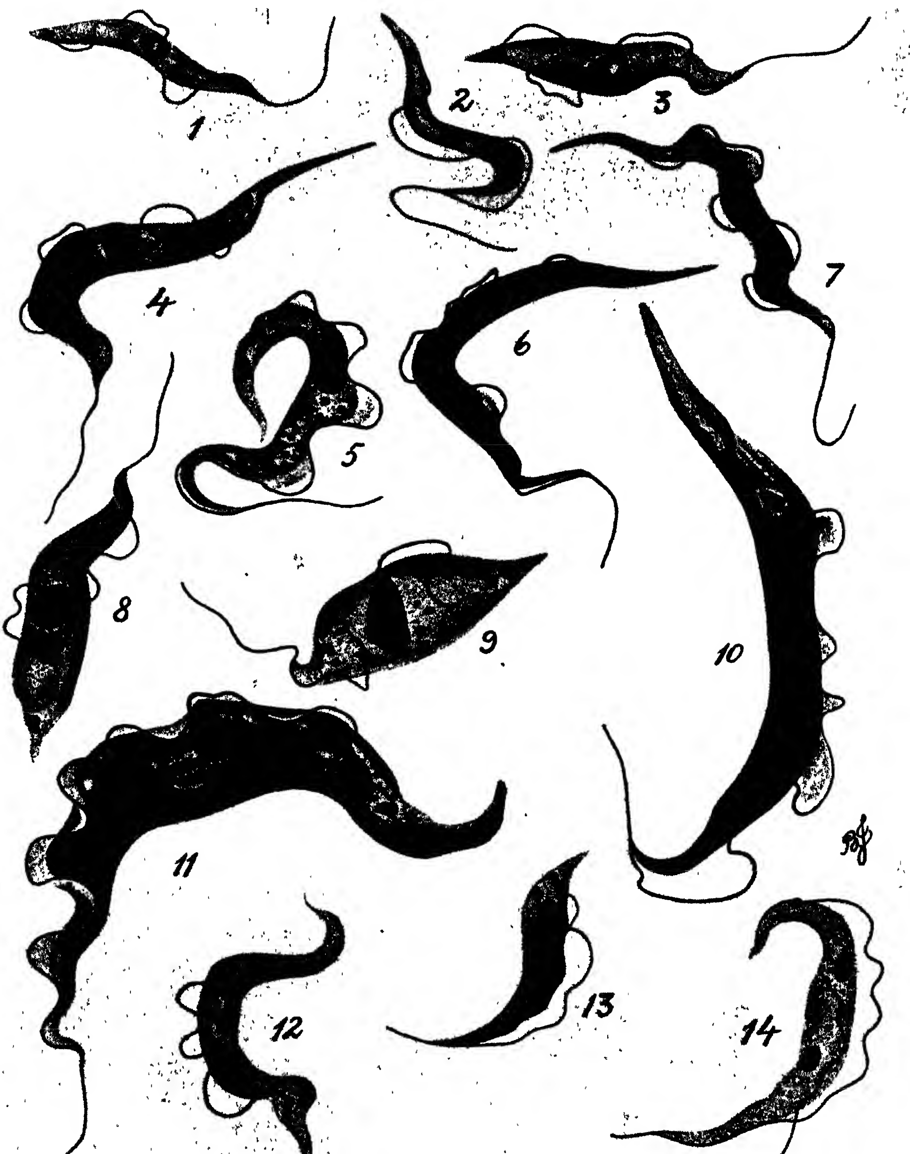


FIG. 235.—TRYPANOSOMES OF BIRDS (×2,000). (1-10, AFTER MINCHIN AND WOODCOCK, 1911; 11, AFTER BRUCE, HAMERTON, BATEMAN, MACKIE, AND LADY BRUCE, 1911; 12-14, AFTER THIROUX, 1905.)

1-10. Various types of *Trypanosoma noctuæ* in the little owl (*Athene noctua*).
11. *Trypanosoma gallinarum* in blood of Uganda fowl.
12-14. *Trypanosoma paddæ* in blood of Java sparrow (*Munia oryzivora*).

some was also inoculable to other birds (*Serinus serinus*, *S. canarius*, *Lagonosticta minima*, *Mariposa phœnicotis*, *Estrilda cinerea*). The infection in canaries was more intense and the mortality higher than in the natural host. These observations serve to indicate the possibility of one species of trypanosome having several hosts (Fig. 235, 12-14).

Multiplication of *T. paddæ* takes place by longitudinal division in the usual manner, but the dividing forms are only seen in the blood during the early stages of an infection. Later no division forms can be found, and in this respect the trypanosome resembles *T. lewisi* of the rat. Examination of the spleen and bone marrow did not reveal a greater number of parasites than the blood. In the case of other bird trypanosomes the bone marrow appears to be the site of election, for they can often be found there when the blood-examination has been negative. For instance, Minchin and Woodcock (1911) noted this in the case of *T. noctuæ* of the little owl, and Woodcock (1910) in *T. fringillinarum* of the chaffinch. These observers pointed out that the trypanosomes were absent from the blood, but were to be found in the bone marrow, especially in winter and spring.

Thiroux succeeded in cultivating *T. paddæ* in blood-agar media.

Avian Trypanosomes in General.

Though a large number of trypanosomes of birds have been given specific names, it is evident that the validity of many of these is very doubtful. Where infections have been studied in any detail, it has been observed that the trypanosomes are very polymorphic.

Morphology. — Minchin and Woodcock (1911) noted a great range in size of *T. noctuæ*, the largest forms being found in the winter and spring (Fig. 235, 1-10). Thus, there are small forms with a total length of 26·5 microns and a breadth of 3·5 microns, intermediate forms measuring 44 to 47·5 microns by 5 to 5·5 microns, and large massive forms 54 to 60 microns by 5·5 to 6 microns. The small forms gradually grow into the large forms, which are found in winter in the bone marrow. It is supposed that in the summer the small forms are reproduced from the large ones by a process of schizogony, but this hypothetical reproductive process was not observed. The small forms reproduce by longitudinal division, and also give rise to certain stout trypanosomes which, according to the authors, are destined to undergo development in the mosquito. The proof that the mosquito, *Culex pipiens*, is the transmitting host of *T. noctuæ*, and that the changes undergone by the trypanosome in the stomach of the mosquito, as described by Woodcock (1914), are true developmental stages, is as yet lacking. It is evident that *T. noctuæ* is markedly polymorphic in the owl, a feature which, if of general occurrence, renders the

identification of species exceedingly difficult, especially as the majority of those which have been named have only been seen in one particular phase in a single blood-film. Furthermore, very little is known as to the number of hosts any trypanosome may infect. As noted above, Thiroux (1905) found that *T. paddæ* was inoculable to a number of different birds. Similarly, Nöller (1920*c*) found that *T. loxiæ* of *Loxia curvirostra* was inoculable to canaries and finches.

Though trypanosomes have been described from many different birds, they can all be referred to one or other of the types described by Minchin and Woodcock as occurring in the cycle of development of the trypanosome of the little owl, *Athene noctua*, and which are illustrated in Fig. 235. As regards the details of their morphology, bird trypanosomes conform to other members of the genus. Nieschulz (1922*a*) has described a rod-shaped structure which occurs in the cytoplasm of cultural forms and a granule which is present on the nuclear membrane. These have already been referred to above (Fig. 154).

Transmission.—As regards the natural transmission of bird trypanosomes very little is known. Schaudinn (1904), and later Woodcock (1914), stated that *C. pipiens* was the intermediate host of *T. noctuæ*. Danilewsky observed that young birds in the nest only a few days old were already infected with trypanosomes. Duke and Robertson (1912) noted that *T. gallinarum* (Fig. 235, 11), first described by Bruce *et al.* (1911*i*) in Uganda fowls, underwent a development in *Glossina palpalis*, resulting in the production of crithidia forms in the stomach. It was concluded, however, that the tsetse fly was not the true host. What is possibly the same trypanosome was seen by Mathis and Leger (1911*a*) in fowls in Tonkin. It is probable that the transmitting hosts of bird trypanosomes will have to be sought amongst the blood-sucking arthropods, which especially infest the nests. There is evidence, however, that bird trypanosomes will develop in mosquitoes. Woodcock (1914) described the changes undergone by *T. noctuæ* in *C. pipiens*. The trypanosomes taken up by the mosquitoes underwent multiplication and became crithidia forms, while finally long slender trypanosomes and very much smaller stumpy trypanosomes were produced. The latter forms bear a resemblance to the metacyclic trypanosomes which are developed in the hind-gut of the flea in the case of *T. lewisi*. Nöller (1920*c*) also noted that *T. loxiæ* underwent a development in *C. pipiens*, as also in *Aëdes argenteus*. This culminated in an accumulation of flagellates in the hind-gut of the mosquitoes. He noted that when *T. loxiæ* and *T. syrnii* were cultivated on blood-agar plates at 18° to 20° C., there was rapid multiplication of crithidia forms, and that a transformation into trypanosomes took place when the plates were incubated at 37° C. In cultures of *T. frin-*

gillinarum, Woodcock (1914) likewise noted that a trypanosome phase followed the appearance and multiplication of crithidia forms. It is evident that the cycle of development of bird trypanosomes in the invertebrate will follow the usual lines, in which crithidia forms first appear, to be followed by metacyclic trypanosomes. No actual transmission by means of mosquitoes or any other invertebrates has as yet been effected. An interesting observation made by Mathis (1914) may be urged in support of the view that the transmitting hosts of bird trypanosomes are mosquitoes. In a species of *Culex* in Tonkin he noted an infection of the salivary glands with flagellates of the crithidia type, and conjectured that these might have been derived from some bird trypanosome (p. 370).

Culture.—That trypanosomes of birds are relatively easy to cultivate in blood-agar media was first demonstrated by Novy and McNeal (1905). Danilewsky (1888), however, had previously observed multiplication of trypanosomes in hanging-drop preparations of bird's blood. Novy and McNeal, and Nieschulz (1922*b*) noted that infections could be demonstrated in birds by the cultural method when blood-examinations were negative. In these cultures the trypanosomes multiply rapidly, becoming transformed into crithidia and rounded or ovoid forms. In older cultures trypanosomes again appear. The cultures may be maintained indefinitely by subculture. Novy and McNeal, and Thiroux (1905) found that birds were with difficulty infected from the cultural forms.

Nöller (1920*c*) and Nieschulz (1922*b*) have cultivated trypanosomes from a number of birds on blood-agar plates. The plates kept at room temperature show mostly crithidia forms. If they are kept at 37° C., the crithidia forms assume the trypanosome structure, but again revert to the crithidia form when the temperature is reduced.

IV. TRYPANOSOMES OF LAND REPTILES INCLUDING CROCODILES.

The first definite record of a trypanosome in a reptile was that of Laveran and Mesnil (1902), who described *T. damoniæ* of the tortoise, *Damonia reevesii*, though as early as 1883 Kunstler had noted a flagellate in the blood of a mud tortoise, and considered it to be allied to the trypanosomes. Since Laveran and Mesnil's discovery, various trypanosomes have been described from crocodiles, tortoises, snakes, and lizards.

In very few cases is the method of transmission actually known, but such information as is available appears to indicate that the trypanosomes of land reptiles, including crocodiles, are transmitted by blood-sucking arthropoda, while those of aquatic reptiles are transmitted by leeches. On this account the trypanosomes of reptiles are considered under two headings.

As regards the trypanosomes which have blood-sucking arthropods as their vectors, it is not definitely known whether the development is in the anterior or posterior station, though the behaviour of *T. kochi* of the crocodile in the tsetse fly is suggestive of a contaminative method of infection.

T. kochi Laveran and Mesnil, 1912.—The crocodile trypanosome was first seen by Minchin, Gray, and Tulloch (1906) in Uganda, but no description was given. Koch (1906) studied it in greater detail, and suggested the possibility that certain flagellates (*T. grayi*, *Herpetomonas grayi*) frequently encountered in tsetse flies had their origin in the trypanosome of the crocodile, on which the flies were noted to feed (see p. 373). As Laveran and Mesnil (1912) have pointed out, Koch did not suppose that the flagellates of the flies, which he thought might have developed from the crocodile trypanosome, were in any way related to *T. gambiense*, though writers have wrongly attributed this view to him. Kleine and Taute (1911) described experiments which gave definite support to Koch's view of the development of the crocodile trypanosome in tsetse flies. In one experiment, thirty-two bred flies (*Glossina palpalis*) were fed on a crocodile, with the result that eleven were found to harbour *H. grayi* when dissected eight to fourteen days later. They believed, however, that the tsetse flies could acquire the flagellates from other hosts than the crocodile. Ross, P. H. (1911), found flagellates of this type in *G. fusca*, while Bruce *et al.* (1914*b*) discovered that both *G. palpalis* and *G. brevipalpis* were liable to be infected with *H. grayi*. They suggested that the flagellates were probably derived from the crocodile, iguana, or some water bird, as both these flies resemble one another in the habit of living near water. Lloyd and Johnson (1924) have found the flagellate in *G. tachinoides* in Nigeria. Kleine (1919*a*) definitely asserts that the flagellate represents developmental forms of the crocodile trypanosome. Roubaud (1912), basing his conclusions on a series of negative feeding experiments and on the fact that Minchin (1907) had described encysted stages of the flagellate in the rectum of the flies, stated that *H. grayi* was a flagellate peculiar to the flies, and was handed on, like other purely insect flagellates, from fly to fly by means of the cysts (Figs. 173 and 220). It is, however, far from clear that the bodies described by Minchin were actually cysts, and it is difficult to understand how tsetse flies, either in the adult or larval stages, could ingest such cysts. It seems probable that Kleine's view is the correct one, in which case the name of the crocodile trypanosome will be *T. grayi* Novy, 1906, and not *T. kochi* Laveran and Mesnil, 1912. Lloyd and Johnson (1924) and Lloyd, Johnson, Young, and Morrison (1924), however, produce evidence that the flagellates of the *H. grayi* type in *G. tachinoides* may be derived from monitors (*Varanus*

exanthematicus) as well as crocodiles on which the flies feed. If this be the case, they would represent developmental forms of *T. varani*, first seen by the writer (1909) in the Sudan. It is possible that under the names *T. grayi* and *H. grayi* several reptilian trypanosomes have been grouped, and that it will not be possible to identify the flagellates first named *H. grayi* with any particular trypanosome. The question of the flagellates of tsetse flies is still further complicated by the recent discovery by Lloyd, Johnson, Young, and Morrison (1924) that crithidia forms indistinguishable from those of *T. grayi* appear in the intestine of *G. tachinoides* after feeding on toads (*Bufo regularis*), which harbour trypanosomes resembling *T. varani* (see p. 374).

The trypanosome (Fig. 236, 3) seen by Bruce *et al.* (1911*f*) in the crocodile (*Crocodilus niloticus*) had a total length of 87 microns, which was made up as follows: posterior end to the kinetoplast, 18 microns; kinetoplast to anterior end, 46 microns; flagellum, 23 microns. The body showed longitudinal myoneme striations. The trypanosome was cultivated by Koch (1906), and by Kleine and Taute (1911). A trypanosome about half the length of the form seen by Bruce was recorded from *C. catepractus* by Dutton, Todd, and Tobey (1907) in the Congo.

Other Trypanosomes of Land Reptiles.

Martin (1907) described as *T. boueti* a broad leaf-like trypanosome from *Mabuia raddoni* of French Guinea, while Bouet (1909) gave the name *T. martini* to a similar form found by him in *M. maculilabris* and *M. perroteti* of the Ivory Coast. França (1911*a*) named the form from the second of these hosts *T. perroteti*. The writer (1909) described as *T. mabuiæ* a trypanosome of *M. quinquetæniata* of the Southern Sudan (Fig. 236, 6-7). The trypanosome occurred in two forms—one a broad leaf-like trypanosome resembling *T. rotatorium* of the frog and measuring 30 to 40 by 8 microns, and the other a smaller trypanosome like *T. inopinatum* of the frog and measuring 20 to 25 by 2 to 2·5 microns. It is possible that the various species described from the skinks of the genus *Mabuia* are different stages of one polymorphic trypanosome like *T. rotatorium* of frogs, in which case Martin's name, *T. boueti*, will have priority. A broad leaf-like trypanosome was described by the writer (1909) as *T. chamæleonis* from *Chamæleon vulgaris* of the Sudan, and another similar form from the monitor (*Varanus niloticus*) as *T. varani*.

Robertson (1908) recorded trypanosomes from two geckos of Ceylon. One which occurred in *Hemidactylus leschenaultii* was named *T. leschenaultii*. It measured 56 to 60 microns in length, and had a flagellum measuring 17 to 20 microns. The other, named *T. pertenue*, occurred in

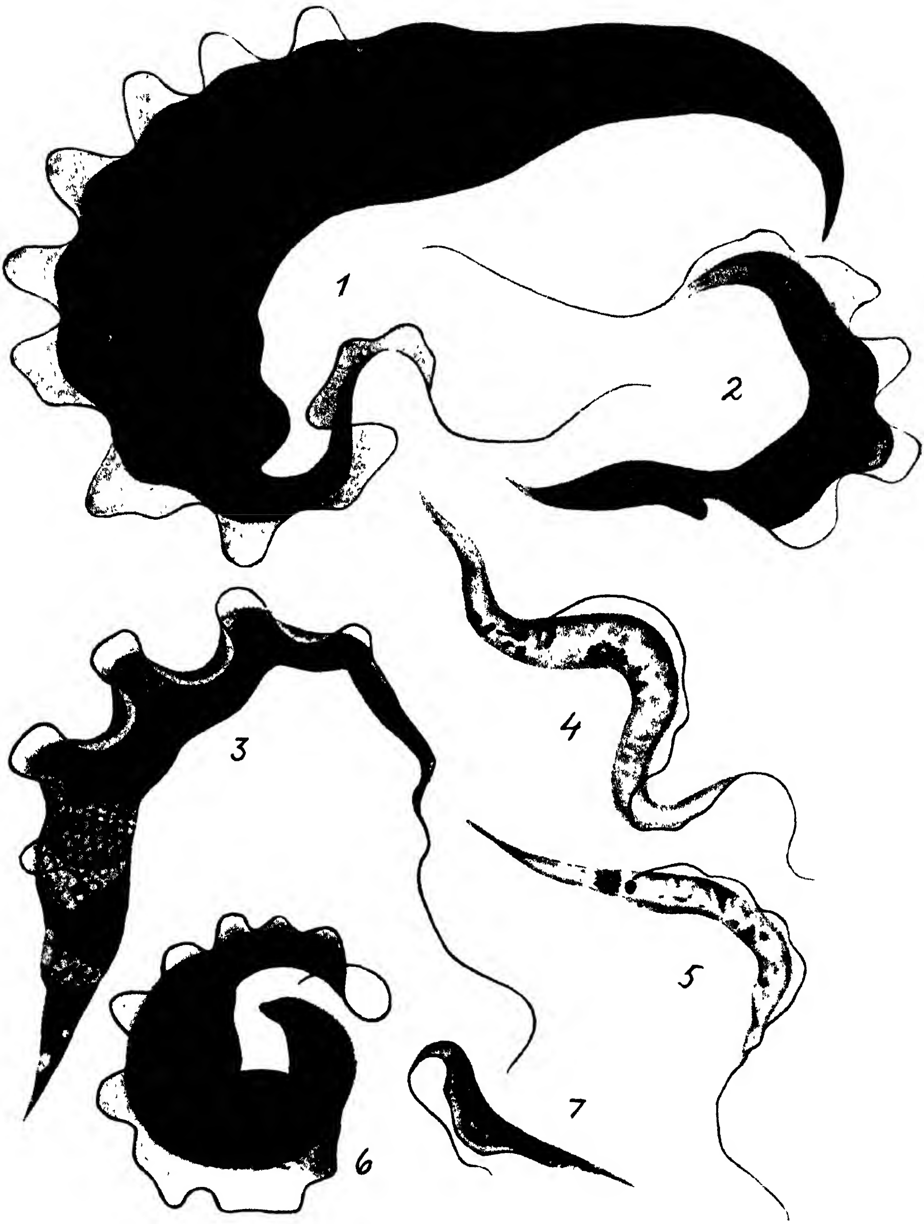


FIG. 236.—TRYPANOSOMES OF REPTILES (×2,000). (1 AND 2, AFTER MATHIS AND LEGER, 1909; 3, AFTER BRUCE, HAMERTON, BATEMAN, MACKIE, AND LADY BRUCE, 1911; 4-7, AFTER WENYON, 1909.)

1 2. *Trypanosoma primeti* of the snake, *Tropidonotus piscator*, of Tonkin.
3. Trypanosome of the Uganda crocodile (*T. kochi* ?).
4 5. *Trypanosoma erythrolampri* of the snake, *Erythrolamprus æsuclapii*, of South America.
6·7. *Trypanosoma mabuiæ* of the lizard, *Mabuia quinquitæniata*, of the Sudan.

H. triedri. It was 30 to 35 microns in length, with a flagellum 15 to 20 microns in length. A similar trypanosome from *Psylodactylus caudicinctus* was named *T. gallyi* by Bouet (1909), while Mathis and Leger (1911) mention the occurrence of another in *Acanthosaura fruhstorferi* in Tonkin Catouillard (1909) gave the name *T. platydactyli* to a trypanosome of *Tarentola mauritanica* of Tunis. It was cultivated in N.N.N. medium by Sergent, Ed. and Et., Lemaire, and Senevet (1914), while Chatton and Blanc (1918*a*) showed that it developed readily in bed bugs fed on the geckos. Todd and Wolbach (1912) mentioned the occurrence of trypanosomes in *Agama colonorum* and *Lygosoma* sp. of the Gambia.

Trypanosomes have been recorded from a number of land snakes. *T. erythrolampri*, seen by the writer (1908) in the South American snake, *Erythrolamprus æsculapii*, is a long narrow form measuring 30 to 34 by 5 to 7 microns (Fig. 236, 4-5). It was found in the blood of a snake which had died in the Zoological Gardens in London, and some of the flagellates had the crithidia structure. It is possible that the crithidia arrangement of nucleus and kinetoplast was the result of changes occurring after the death of the host. The writer (1909) gave the name *T. najæ* to a trypanosome of the Sudan cobra (*Naja nigricollis*). It measured 50 microns in length, and was only seen in the living condition. Bouet (1909) described as *T. clozeli* a large broad trypanosome of the African snake, *Tropidonotus ferox*. The nucleus and kinetoplast were close together near the middle of the body, which measured about 100 to 106·5 by 10 to 25 microns. Dutton, Todd, and Tobey (1907) record a trypanosome from the puff adder, *Bitis arietans*, of the Gambia. According to Johnston and Cleland (1910), Love discovered a trypanosome in the Australian snake, *Diemenia textilis*.

V. TRYPANOSOMES OF AQUATIC VERTEBRATES TRANSMITTED BY LEECHES.

1. Trypanosomes of Aquatic Reptiles.

The best-known trypanosomes of this group are those of aquatic chelonians. The development of *T. vittatæ* has been studied by Robertson (1908).

Trypanosoma vittatæ Robertson, 1908.—This trypanosome (Fig. 237) was discovered in the soft tortoise (*Emyda vittata*) of Ceylon by Robertson (1908), who studied it not only in the vertebrate host, but also in the leech (*Glossosiphonia* sp.), which is probably the invertebrate vector of the trypanosome. Development, however, was found to take place also in the horse leech, *Pœcilobdella granulosa*.

In the blood of the tortoise (Fig. 237, 1-4), the largest trypanosomes have a body measuring between 60 and 70 microns in length and 8 to 9

FIG. 237.—*Trypanosoma vittatæ*, PARASITE OF THE CEYLON TORTOISE, *Emyda vittata*, AND THE LEECH, *Pœcilobdella granulosum* (× 1,600). (AFTER ROBERTSON, 1909.)

1-4. *T. vittatæ* in blood of tortoise.
5-6. Division stages of rounded-off trypanosome in intestine of leech.
7. One of the products of division.
8. Elongation of body to form crithidia stage.
9-10. Division of elongating forms.
11-13. Trypanosome forms evolved from the crithidia forms

microns in breadth. The shortest forms were about 25 microns long and 4 to 5 microns broad. Intermediate forms also occurred. The undulating membrane is markedly frilled. The flagellum measures up to 25 microns in the large and small trypanosomes, being relatively longer in the latter. In the large forms the body is seen to be longitudinally marked by parallel lines, an indication of myonemes. When examined in the fresh condition, the trypanosome is seen to writhe about locally with little progression. Occasionally there is a slow translatory movement, the trypanosome revolving spirally on its axis. In the blood of the tortoise division stages were rarely found, and these only in the case of trypanosomes of the intermediate size. It is possible that active multiplication only takes place in the early stages of an infection, or is chiefly confined to the internal organs.

In the crop of the leech a cycle of development takes place, resulting in the formation of crithidia forms (Fig. 237, 5-10). The earliest stage of this cycle consists in the rounding-off of the large trypanosomes, a process which can be studied in fresh blood-preparations under the microscope. The large trypanosomes become retracted in various ways to form globular masses of cytoplasm. The myonemes cease to be visible, as also the nuclei. The axonemes become detached from the membranes, and finally disintegrate. These globular bodies then commence to divide. By two divisions four pyriform bodies are produced from each, and these remain more or less attached to one another while they form flagella. The latter first appear as short rods, which increase in length till their full size is reached. These flagellate bodies, when stained, are found to have the crithidia structure. By their further multiplication the crop of the leech becomes populated with a large number of long, slender, and very actively motile crithidia forms, which eventually give rise to metacyclic trypanosomes (Fig. 237, 11-13). The development appears to be limited to the crop, and the exact mechanism of the infecting process was not elucidated. The leech, *Glossosiphonia* sp., is peculiarly suited to play the part of an intermediate host, as it has the habit of wandering from one tortoise to another.

Other Trypanosomes of Aquatic Reptiles.

Trypanosoma damoniæ, the first trypanosome to be described in a reptile, has been mentioned above. It was discovered by Laveran and Mesnil (1902). The length was 32 microns, of which the flagellum formed about one-third, and the breadth 4 microns. Trypanosomes have been met with in a number of other chelonians. Dutton and Todd (1903) and Dutton, Todd, and Tobey (1907) noted the presence of trypanosomes in tortoises of the Gambia, as did Minchin (1910) in one in Uganda. Bouet (1909) gave the

name *T. pontyi* to a trypanosome of the tortoise, *Sternotherus derbianus*, of Africa. *T. chelodina* was recorded from *Chelodina longicollis* by Johnson (1907), and what is probably the same form from *Emydura krefftii* by Johnston and Cleland (1910, 1912), who saw it also in *C. longicollis*.

In aquatic snakes trypanosomes also occur. Mathis and Léger (1909*a*) gave the name *T. primeti* to a trypanosome discovered by them in *Tropidonotus piscator* and *Hypsirhina chinensis* (Fig. 236, 1-2). There occur large forms measuring 105 by 14 microns and small forms measuring 57 by 7 microns. Brumpt (1914*a*) saw a trypanosome which he named *T. brazili* in the Brazilian water snake, *Helicops modestus*. He demonstrated a complete development terminating in metacyclic trypanosomes in the leeches, *Placobdella brasiliensis* and *P. catenigera* (Fig. 452). The whole of the development was confined to the stomach, no infection of the proboscis sheath occurring even after several months. Brumpt suggests the possibility of snakes becoming infected by swallowing the leeches. In the case of another snake (*Rhadmœa merremii*), specimens of the leech, *P. brasiliensis*, which had been allowed to feed on it were later found to contain developmental stages of a trypanosome. The snake was then carefully examined, and found to have a small infection of a trypanosome resembling *T. brazili*.

2. Trypanosomes of Amphibia.

(*a*) Trypanosomes of Anura.

Gluge (1842) appears to have been the first to have seen what was probably a trypanosome in the blood of the frog. In the following year Mayer described various forms of the same organism under the names of *Amœba rotatorium*, *Paramecium loricatum*, *P. costatum*, while later in the year Gruby gave a better description, and suggested for it the new name *Trypanosoma sanguinis*. The trypanosome was seen by other observers, and Lieberkühn (1870) proposed the name *Monas rotatoria* and Ray Lankester (1871) the name *Undulina ranarum*. Grassi (1881-1882) studied the trypanosome in various frogs and toads, and separated from *T. sanguinis* the forms which, though possessing a membrane, appeared to have no flagellum, under the name of *Paramecioides costatus*. These were evidently the forms studied by Mayer, and named by him *Paramecium costatum*. The trypanosomes of frogs and toads were then studied by various observers in many parts of the world, and owing to their extreme polymorphism, numerous names appeared which are undoubtedly synonyms.

Nöller (1913*b*) has studied the whole question, and has come to the conclusion that only two certain species are represented amongst the large number of trypanosomes described from frogs and toads—viz., *T. rotatorium* (Mayer, 1843), and *T. inopinatum* Sergent, 1904.

Trypanosomes conforming to one or other of the types seen in *T. rotatorium* have been described from frogs and toads from various parts of the world, but it is not possible definitely to assert that they all belong to one species, though in many cases this is highly probable (Fig. 238). The following names, which may be synonyms

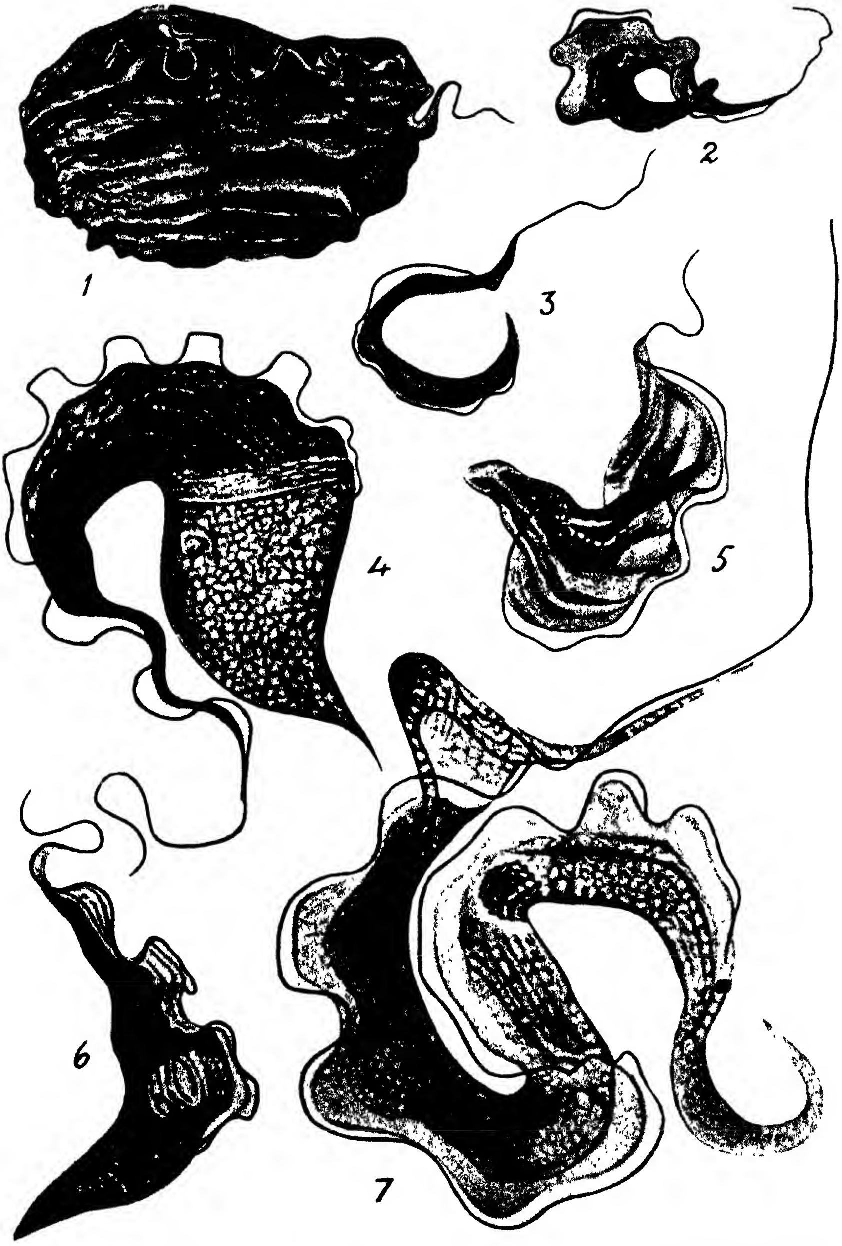


FIG. 238.—TRYPANOSOMES SEEN IN THE BLOOD OF CONGO FROGS (×2,000). (AFTER DUTTON, TODD, AND TOBEY, 1907.)

They were described under the following names: 1-3 and 5, *T. loricatum*; 4 and 6, *T. mega*; 7, *T. karyozeukton*.

of *T. rotatorium*, have been used for trypanosomes of frogs and toads, apart from those already given: *T. mega* Dutton and Todd, 1903; *T. karyozeukton* Dutton and Todd, 1903; *T. rotatorium* var. *nana* Ed. and Et. Sergent, 1905; *T. nelspruitense* Laveran, 1905; *T. belli* Nabarro, 1907; *T. borelli* Marchoux and Salimbeni, 1907; *T. hylæ* França, 1908; *T. leptodactyli* Carini, 1907; *T. innominatum* Pittaluga, 1905; *T. somaliense* Brumpt, 1906; *T. bocagei* França, 1911; *T. bocagei* var. *parva* and *magna* Mathis and Leger, 1911; *T. chattoni* Mathis and Leger, 1911; T. *tumida* Averinzev, 1918.

Similarly, in the case of *T. inopinatum* the following names appear to be synonyms: *T. undulans* França and Athias, 1906; *T. elegans* França and Athias, 1906; *T. hendersoni* Patton, 1908.

Laveran and Mesnil (1912) arrange the trypanosomes of frogs and toads in four groups. They separate from the two species named above *T. leptodactyli* of *Leptodactylus ocellatus* of Brazil and all the trypanosomes of toads. França (1925) believes that *T. mega* and *T. karyozeukton* of *Bufo regularis* are good species.

Trypanosoma rotatorium (Mayer, 1843).—As a result of the work of Nöller (1913*b*), it would appear that this trypanosome is primarily a parasite of the tadpole, and is handed on from one tadpole to another by the leech, *Hemiclepsis marginata*. In the tadpole, and also in young frogs, the flagellate is of the usual narrow trypanosome type (Fig. 239, 1-2). In older frogs there appear many remarkable forms which are to be regarded as derived by overgrowth from the more typical trypanosomes of the tadpole (Fig. 239, 11-12).

Morphology.—The tadpole trypanosome, according to Nöller (1913*b*), has a body measuring from 25 to 35 microns in length. The nucleus lies at the centre of the body, and is 2 to 2·8 microns in diameter. It is spherical, and in properly fixed specimens is seen to have a central karyosome. The flagellum is 12 to 15 microns in length. The posterior end of the body is sharply pointed and the undulating membrane is well developed. Trypanosomes first appear in the tadpole five or six days after exposure to infection by the leech. The first trypanosomes to appear are small and narrow, and it is about the tenth day after exposure that the infection reaches its height, and the more typical trypanosomes corresponding with the measurements given above appear. They are, however, not numerous, as only about twenty occur in a square (18 by 18 mm.) cover-glass preparation of the blood. Reproduction takes place in the usual manner by longitudinal division. Nöller was unable to discover any intracellular forms or stages of multiple division as described by Machado (1911), nor was he able to obtain any evidence justifying the separation of any of these trypanosomes into male and female individuals, as this observer has done.

The trypanosomes of the adult frog occur in three main types:

1. Long narrow forms with well-developed undulating membrane, spherical nucleus, compact kinetoplast, sharp-pointed and drawn-out posterior end, and flagellum. The periplast may be longitudinally marked.

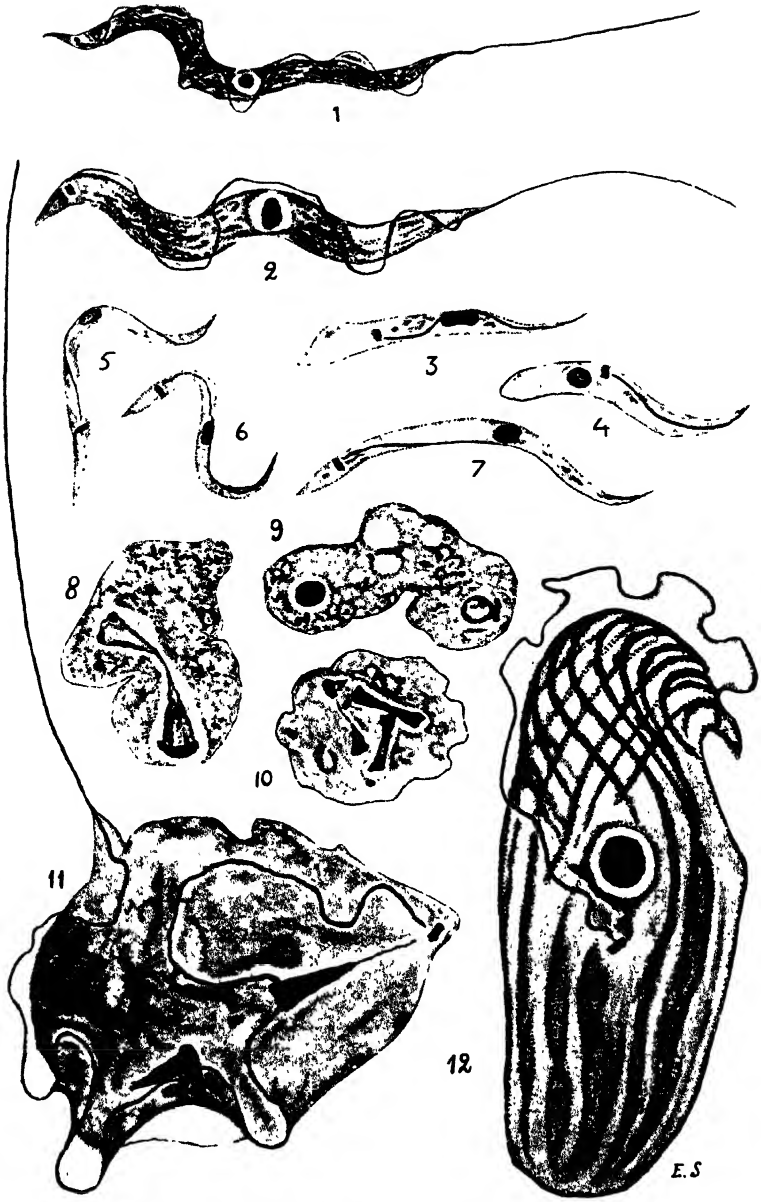


FIG. 239.—*Trypanosoma rotatorium* PARASITIC IN THE FROG AND THE LEECH (*Hemiclepsis marginata*) (×2,900). (AFTER NÖLLER, 1913.)

1-2. Typical forms in the blood of the tadpole. 3. Crithidia forms from stomach of leech.
4-7. Trypanosome forms from stomach of leech.
8-10. Nuclear divisions of rounded-off forms which develop in cover-glass cultures of the large striated forms from frog's blood.
11. Flat leaf-like form from frog's blood. 12. Solid striated form from frog's blood.

2. Large compact individuals (Fig. 239, 12), more or less spherical or ovoid in shape, with a longitudinally striated periplast, spherical nucleus, and spherical kinetoplast which lies near the nucleus. The undulating membrane is well developed, while the axoneme usually terminates at the anterior end. In some, however, there is a short flagellum. The posterior end of the trypanosome is often rounded.

3. Flat leaf-like forms (Fig. 239, 11), with rounded or pointed posterior end, well-developed undulating membrane, and long flagellum. The periplast is usually not striated. The nucleus is a long drawn-out structure, the posterior end of which lies near the kinetoplast at the posterior end of the trypanosome, while the other end terminates at the middle of the body.

According to Nöller, it is in the order given above that these forms appear in the blood of the frog. Those of type 1 are undoubtedly developed by growth from the tadpole form, which is of the same shape but smaller. By a further growth and thickening the large solid forms of type 2 are produced. Whether the leaf-like forms of type 3, with their curious elongate nuclei, are developed from the solid forms in some way or from those of type 1 through an increase in breadth and not in thickness cannot be stated with certainty, but the latter would seem to be more probable. The three types are not sharply marked off from one another, as connecting links occur. It is thus evident that *T. rotatorium* of the tadpole and frog exhibits a great variety of shape and form, and it is for this reason that numerous synonyms have arisen.

Susceptibility of Frogs and Other Animals.—Nöller (1913*b*, 1917), working in Europe, has published accounts of inoculation experiments performed with the trypanosome of frogs. The blood of tadpoles of *Rana esculenta* infected with trypanosomes was inoculated into adult frogs, which developed a larger infection of the forms characteristic of frogs than they had before. Further inoculations were carried out with large doses of cultural forms from blood-agar plates. Though the frogs had already a small infection, they developed an enormous one which killed them. The blood and organs were swarming with the large trypanosomes, and this was especially marked in the kidneys, where veritable emboli of these forms occurred. These infections were undoubtedly superimposed on old-standing ones. Inoculation of *R. temporaria*, which is rarely found naturally infected, with cultures of *T. rotatorium* derived from *R. esculenta* led to a milder blood infection though the kidneys were found heavily loaded with trypanosomes. The tree frog, *Hyla arborea*, was also infected, a fact which suggests that *T. hylæ* of França (1908*d*) is actually *T. rotatorium*. Two toads, *Bombinator igneus*, were inoculated with very large doses of culture, and no infection took place. This species of toad has never been found naturally infected with a trypanosome. Similar

experiments with the tortoise and goldfish gave only negative results. It is possible that *T. rotatorium* occurs in a number of different hosts.

Transmission.—The intermediate host of *T. rotatorium*, as first demonstrated by França (1908*a*), and then by Nöller (1913*b*), is the leech, *Hemiclepsis marginata* (Fig. 240), but before discussing the development in this invertebrate it will be necessary to describe some details of its anatomy. The mouth opens into the proboscis, a thick-ridged cylinder which is armed with teeth, and completely retractable into the proboscis sheath (Fig. 244). The sheath is an infolding of the anterior end of the body, forming a cavity in which the proboscis lies. Through the anterior opening of the proboscis sheath the proboscis can be protruded at the time of feeding, while the margin of the proboscis sheath is applied to the skin. It will thus be seen that in the act of feeding the contents of the proboscis sheath can gain easy access to the wound made by the proboscis. An œsophagus leads from the proboscis to the stomach or crop, a large structure with lateral diverticula occupying most of the body cavity. From the stomach an intestine with diverticula at its anterior end leads to the anus.

On account of the scanty infection in the tadpole, it was impossible to observe the earliest stage of development in the leech. In two or three days after feeding on the infected tadpole there are present in the stomach stumpy forms which were described as having the leptomonas structure, but which may in reality be crithidia (Fig. 239, 3).

FIG. 240.—*Hemiclepsis marginata*, A TRANSMITTER OF TRYPANOSOMES OF FROGS AND FISH (× 3). (AFTER HARDING, 1910.)

How these actually arise was not determined. They may have been the result of repeated binary fissions of the ingested trypanosomes, or, perhaps, what is more probable, the products of the segmentation of a spherical stage such as Robertson (1907, 1909, 1909*a*) and Brumpt (1905) have described in the development of fish trypanosomes in leeches (see p. 603). Nöller has observed such a multiplication of the large thick individuals in cultures made from frog's blood (Fig. 239, 8-10). From the third day onwards there begin to appear very active narrow crithidia forms. At the end of a week narrow trypanosomes occur, and they gradually replace the other forms (Fig. 239, 4-7).

Towards the end of the period of digestion (ten to fourteen days) the trypanosomes migrate forwards to the proboscis, and pass out of the mouth

into the proboscis sheath, where they multiply rapidly. Infection takes place from the proboscis sheath during the sucking act. After feeding, the leech has emptied its proboscis sheath and multiplication of the trypanosomes commences again in the stomach, and reinfection of the proboscis sheath again occurs towards the end of digestion. The development in the leech takes place in the stomach alone, the intestine being free from flagellates.

No intracellular stage was observed in the leech, nor did the trypanosomes invade the body cavity. As Nöller points out, it is remarkable how easily the young leeches infect themselves from tadpoles which have a very scanty infection of trypanosomes. Over a hundred young leeches were thus infected. On the other hand, twenty-six leeches had a full feed on an adult frog, in the blood of which occurred the large solid giant forms as well as the thin leaf-like ones. Not a single leech was infected, though they were kept under the same conditions as regards temperature as the others (10° to 20° C.). It might be urged that the trypanosomes of the adult frogs belonged to a different species from that of tadpoles, but this view is not tenable, as occasionally the young frogs show the typical tadpole forms, while the frogs raised from the tadpoles only showed the larger forms. For these and various other reasons, Nöller concludes that the typical tadpole forms become transformed into those which appear in the frogs on account of change in the character of the blood associated with the metamorphosis of the tadpole into the frog.

Culture.—*T. rotatorium* is readily cultivated in blood-agar medium. Of special interest is the behaviour of the large trypanosomes *in vitro*. The phenomenon was first observed by Danilewsky (1885*a*, 1889) and Chalachnikov (1888), and was later observed by Mathis (1906), França and Athias (1907), Dutton, Todd, and Tobey (1907), Lebedeff (1910), Doflein (1910), and lastly by Nöller (1913*b*). It consists of the rounding-off of the trypanosome and its segmentation into a number of small individuals. The accounts differ somewhat in details, the following being based on the work of the last-named author. A drop of blood from a frog was diluted with a similar quantity of bouillon, and a moist preparation made and sealed to prevent drying, and examined at ordinary laboratory temperature (10° to 25° C.). If one of the large solid striated trypanosomes (type 2) is kept under observation, it will be found to lose its membrane and flagellum. Furthermore, the longitudinal markings disappear, and, owing to various fibrous structures which appear adherent to the cytoplasmic mass, it seems as if the striated periplast is thrown off. Nuclear division can be seen to take place, and finally after about five to six hours the cytoplasm divides, the two daughter individuals remaining side by side. Each daughter then divides again, and the process is

repeated till a collection of twenty to thirty-two small cytoplasmic bodies devoid of flagella results. After twenty to twenty-four hours from the commencement the small bodies begin to exhibit trembling movements, and careful observation reveals a short flagellum on each. Division of these forms continues, and they gradually elongate and assume the crithidia form with a short undulating membrane. After forty-eight hours each original trypanosome will have given rise to a cluster made up of about 150 small crithidia forms. The clusters then break up, and the individuals swim away and continue their multiplication. It will be noted that the original trypanosome loses its flagellum entirely, and those of the daughter individuals are newly formed. Furthermore, the division is always a repeated simple binary fission, and not a multiple segmentation. Nöller was able to make a similar observation on the broad leaf-like trypanosomes (type 3). In the case of these the process proceeds more slowly, and the large cytoplasmic body formed from the original trypanosome extrudes a number of bud-like processes representing the daughter individuals, which, however, do not detach themselves. As many as forty-eight may be present after forty-eight hours. After another twenty-four hours flagella develop at the pointed extremities of these processes, and finally a mass of small crithidia forms is produced. They remain grouped together, however, for a much longer time than in the development of the trypanosome of type 2. Nöller seems to think that the length of time that these daughter forms remain together in clusters is suggestive of the division having taken place within the periplast of the original trypanosome. It is probable that this development, which takes place *in vitro*, represents the early development in the leech, *Hemiclepsis marginata*. Nöller believes that the leech, *Piscicola geometra*, will also prove to be a vector of *T. rotatorium*.

Ponselle (1923*b*) has shown that this development of the large trypanosome is directly dependent upon the reaction of the medium. It will not take place in blood mixed with simple saline solution, but occurs if broth is used to dilute the blood. Broth having an acid reaction (about pH 6·3), he tested saline to which 0·2 per cent. HCl was added, and found that the development took place. By substituting a more complex mixture such as Ringer-Locke solution for the simple saline, the development was even quicker. It was found that cultures of *T. rotatorium* could easily be obtained in a mixture of broth and one-tenth its volume of defibrinated rabbit's blood. No development occurred in a mixture of equal parts of distilled water and defibrinated rabbit's blood. In the case of *T. inopinatum* development readily occurred in the latter, but not in the former, so that each mixture appears to be specific for its particular trypanosome.

Trypanosoma inopinatum Ed. and Et. Sergent, 1904.—This trypano-

some, like *T. rotatorium*, is a parasite of the edible frog, *Rana esculenta*, and has been described under a variety of names owing to the fact that the blood form changes considerably during the course of an infection.

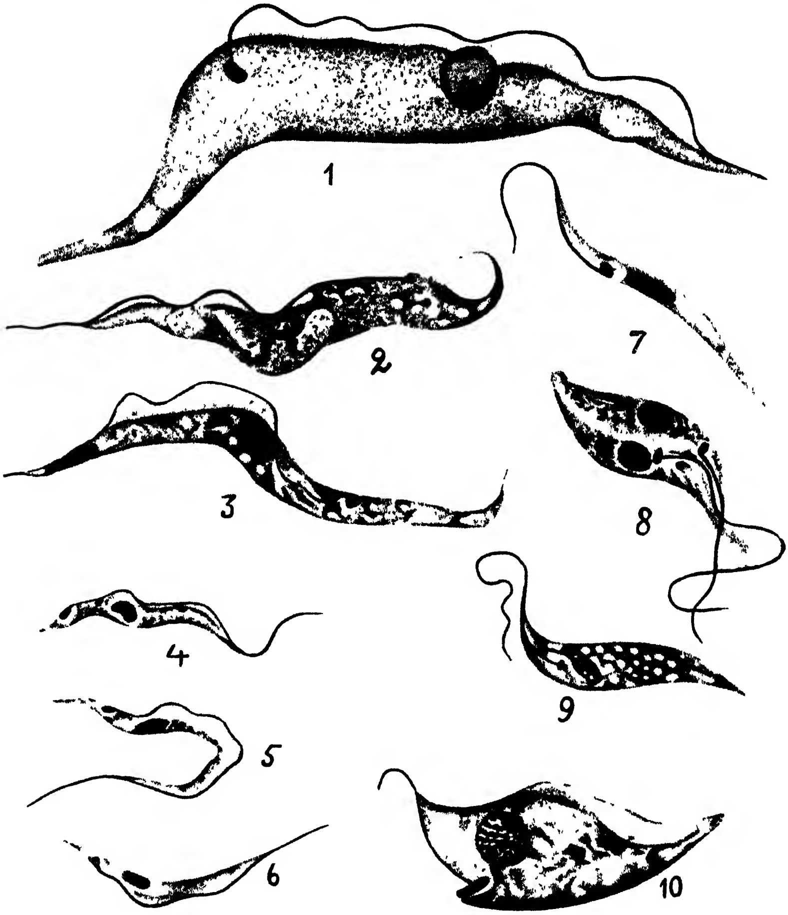


FIG. 241.—*Trypanosoma inopinatum* OF THE FROG (×2,000). (AFTER FRANÇA, 1915.)

1. Fully-grown form (*T. undulans*). 2-3. Forms of intermediate size (*T. elegans*). 4-6. Typical small forms (*T. inopinatum*). 7-10. Cultural forms.

It was originally named *T. inopinatum* by its discoverers in Algiers, then other stages were named *T. undulans* and *T. elegans* by França and Athias (1906) in Portugal, and what is probably the same form in *R. tigrina*, *T. hendersoni* by Patton (1908*c*) in India. Observations by Brumpt (1906*c*) and França (1909, 1911*b*, 1915), who followed the infection in frogs from its beginning, conclusively demonstrated that all these forms are merely stages of development of one and the same organism (Fig. 241).

Morphology.—The first trypanosomes to appear in a frog after inoculation are small trypanosomes with a body 16·5 to 21 microns in length by 1·5 to 2·2 microns in breadth. The posterior end of the body is pointed, while the margin of the undulating membrane is fairly straight. The flagellum is 6 to 10·5 microns in length. The nucleus is central and the kinetoplast well developed. These are the forms seen by the original observers, who noted that the trypanosome bore a striking resemblance to the late phase form of *T. lewisi* of the rat (Fig. 241, 4-6). After a few days, during which the small forms alone are present in the blood, larger forms begin to appear by growth of these. They measure 35 to 36 microns in length and 2·2 to 3·5 microns in width. These are the trypanosomes which were originally described as *T. elegans* (Fig. 241, 2-3). The flagellum is only 5 to 6 microns in length. By continued growth they give rise to still larger forms (*T. undulans*) 36 to 37 microns by 4·5 microns (Fig. 241, 1). Sometimes much larger forms up to 54 microns in length occur. About a month after the infection first appeared the large forms may be the only ones present in the blood.

Apparently none of these various types of trypanosome seen in the blood is undergoing division. França (1915), in smears of the lung, has noted within the cells leishmania forms, many of which are in process of division. Between these and the small trypanosomes every intermediate stage can be traced. It would thus appear that reproduction takes place by division of leishmania forms in the lung or other organs in much the same way as occurs in *T. cruzi*. The leishmania forms are apparently derived in the first place from the flagellates inoculated by the leech.

Transmission.—Billet (1904), who found a variety of flagellates in the intestine of the leech (*Helobdella algira*), which fed upon the frog, came to the conclusion that it was the transmitting host of the trypanosome (Fig. 242). Accordingly, Brumpt (1906*c*) in Paris obtained a number of these leeches from Algiers. He found that *Rana esculenta* of France was easily infected by the bites of the leeches, the first trypanosomes appearing in their blood in eight to ten days. The European frog, moreover, was very susceptible, for in many cases the infection proved fatal. The trypanosomes were found to be present in enormous numbers, the heart of frogs which had died being filled with an embolus of the organisms. *R. temporaria* is also susceptible to the infection. França (1915, 1920) notes that the heaviest infections follow inoculation with the young forms of the trypanosome.

The process of development in the leech has been described by Brumpt (1906*c*). The large form of the trypanosome is the one taken up by the leech, and in the stomach it gives rise to numbers of crithidia forms. This process does not seem to have been described in detail, but from

observations under the cover-glass in wet films França has noted that the large trypanosomes became more rounded and that multiplication of the nuclei and kinetoplasts takes place till several pairs are present. The cytoplasm segments into a corresponding number of crithidia forms after axonemes have grown out from the kinetoplasts. It is probable also that the large forms divide unequally and repeatedly, giving rise to small crithidia forms with gradual reduction in size of the parent. In whatever manner the process takes place in the leech, the stomach soon becomes crowded with large numbers of these crithidia forms, which multiply by fission in the usual manner. Eventually a return to the trypanosome type is noted, and it is these forms which probably, by migration along the œsophagus to the opening of the proboscis, gain access to the cavity of the proboscis sheath, where they accumulate, and are transmitted to the frog when the leech feeds. In the case of this trypanosome of the frog, as with the others which have been considered above, the trypanosomes taken up by the invertebrate become at first crithidia forms, which later are transformed into trypanosomes again. These metacyclic trypanosomes which appear at the end of the cycle reproduce the infection in the vertebrate.

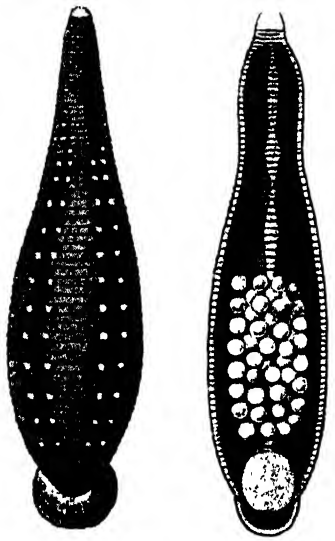


FIG. 242. — *Helobdella algira* (×5), THE TRANSMITTER OF *Trypanosoma inopinatum*. (AFTER BRUMPT, 1922.)

Dorsal view and ventral view with attached eggs.

In connection with the transmission of *T. inopinatum* by the leech, *H. algira*, Brumpt (1907) noted what is interpreted as a definite hereditary transmission in the leech. The embryos, when they hatch from the egg, attach themselves to the ventral surface of the parent leech, and in this position were found by Brumpt to be infected. He has been able to observe this infection persisting through five successive generations of leeches. The infected young are able to infect frogs. Brumpt considers that the egg is infected while still within the parent, but he does not seem to have excluded the possibility of the young leeches being infected soon after hatching from flagellates which escape into the water from the intestine of the parent. He does not state whether young leeches removed from the egg are already infected or not.

Culture.—Ponselle (1923) has shown that *T. inopinatum* is readily culturable in a mixture of equal parts of defibrinated rabbit's blood

inactivated at 56° C. for thirty minutes and distilled water. In this mixture *T. rotatorium* will not develop.

According to Brumpt (1914*a*), *T. leptodactyli* of the Brazilian frog, *Leptodactylus ocellatus*, undergoes a complete development, terminating in the appearance of metacyclic trypanosomes in the proboscis sheath, in the leech, *Placobdella braziliensis*.

Lloyd, Johnson, Young, and Morrison (1924) have shown that laboratory bred *Glossina tachinoides* in Nigeria develop a crithidial infection of the intestine after feeding on toads, *Bufo regularis*, which harbour trypanosomes resembling *T. varani*.

(*b*) Trypanosomes of Urodeles.

A trypanosome in an American newt (*Diemyctylus viridescens*) was described by Tobey (1906) under the name of *T. diemyctyli*. The body of the trypanosome measures 45 to 50 microns, and there is a flagellum 24 microns in length. The breadth varies from 2 to 5 microns. The undulating membrane is well developed. Hegner (1921) has called attention to the frequency with which these newts are infected. Ogawa (1913) described a trypanosome named by him *T. tritonis* from the Japanese newt, *Triton pyrrhogaster*. It measures 57 to 80 microns in length by 2·4 to 6·4 microns in breadth. The flagellum is about 15 microns long. It was readily cultivated in bouillon to which a tenth part of defibrinated rabbit's blood had been added.

3. Trypanosomes of Fish.

The trypanosomes of fish have attracted attention since Valentin's discovery of what was either one of these flagellates or a trypanoplasm in the blood of a trout (*Salmo fario*) in 1841. A large number have since been seen in both fresh and salt-water fish in various parts of the world, and many of them have been given specific names. In some cases there is evidence that one and the same trypanosome may have several hosts.

Morphology.—The trypanosomes of fish usually have long and narrow bodies (Fig. 243). When observed alive, they wriggle about in a peculiar snake or worm-like manner, and frequently roll themselves into knots, only to extend themselves again. The trypanosome of a ray (*T. giganteum*) may be as much as 130 microns in length, while that of the pike (*T. remaki*) may have a body only 15 microns long. The majority of forms are about 50 microns in length, with a breadth of 2 to 5 microns. There is a flagellum of varying length and a well-developed undulating membrane. The kinetoplast is generally large, and the nucleus, which is often easily visible in the living trypanosome, is centrally placed. Sometimes, as in the case of *T. remaki*, two types of trypanosome are present in the blood

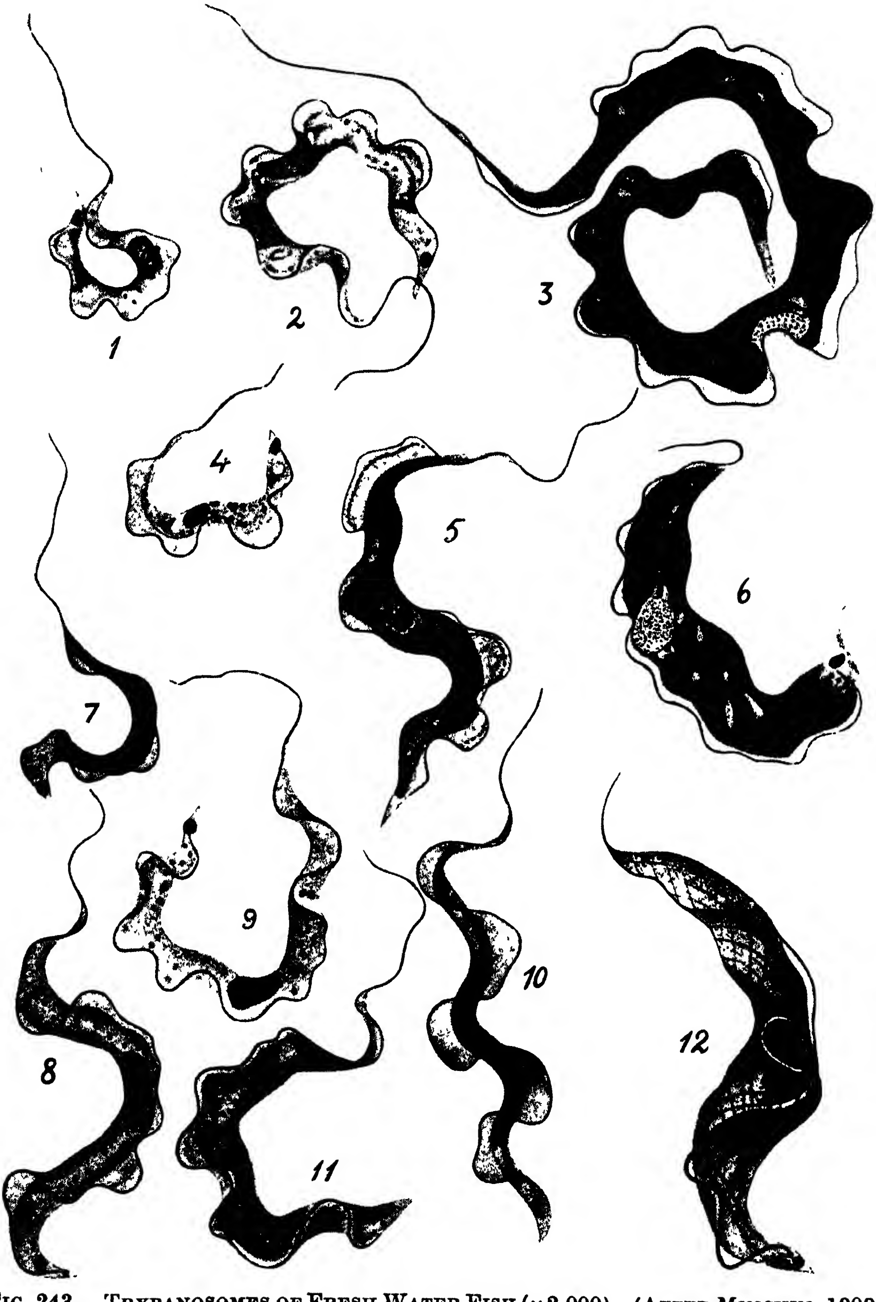


FIG. 243.—TRYPANOSOMES OF FRESH WATER FISH (× 2,000). (AFTER MINCHIN, 1909.)

1-3. *Trypanosoma granulosum* of the eel.
4-6. *T. percæ* of the perch.
7-8. *T. remaki* of the pike.
9-10. *T. tincæ* of the tench.
11. *T. abramidis* of the bream.
12. *T. percæ*, showing myonemes.

(Fig. 243, 7-8), as pointed out by Laveran and Mesnil (1901*c*) and Minchin (1909). There are large forms (*T. remaki* var. *magna*) measuring 45 to 57 microns in length, of which nearly 20 microns is taken up by the flagellum, and small forms (*T. remaki* var. *parva*), which may have a body 10 to 25 microns in length with a flagellum from 10 to 17 microns long. It seems

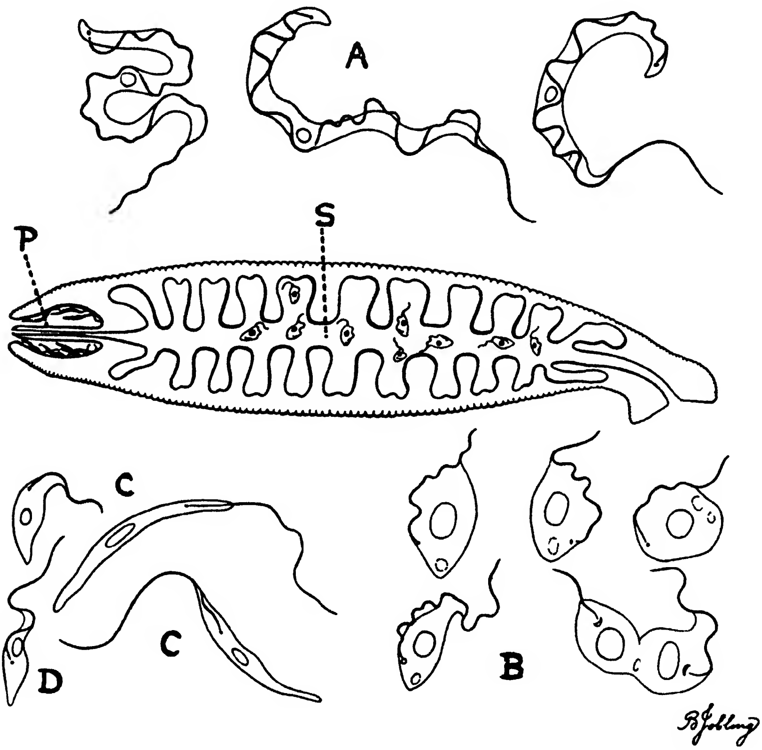


FIG. 244.—DIAGRAM OF TRYPANOSOMES IN THE BLOOD OF A FISH AND IN THE LEECH. (AFTER WENYON, 1922.)

A. Trypanosomes in blood of fish. B. Developmental forms in stomach of leech.
S. Trypanosomes in stomach of leech. P. Developmental forms in proboscis sheath.
C. Crithidia forms in proboscis sheath of leech.
D. Metacyclic trypanosomes in proboscis sheath of leech.

hardly probable, however, that the forms seen in the pike belong to two species, though Minchin (1909) asserts that the two types are sharply marked off from one another.

Some of the larger trypanosomes of fish, as, for instance, *T. percæ* Minchin, 1909, of the perch, may have long longitudinal myonemes well developed (Fig. 243, 12).

Susceptibility of Fish.—Trypanosomes of fish are directly inoculable from one to another. Thus, Laveran and Mesnil (1904) state that they

had been able to infect pike and eels by injecting blood from infected fish. Very few attempts, however, have been made to infect fish with trypanosomes from other species. Lebailly (1906) made some experiments of this nature without success. Robertson (1911) found that the trypanosome of the goldfish, perch, and bream could be transmitted to goldfish by the leech.

Transmission. — In nature, the trypanosomes of fish are carried by leeches (Fig. 244). Some attempts by Minchin (1909) to infect the crustacean *Argulus* by placing them on fish gave no result.

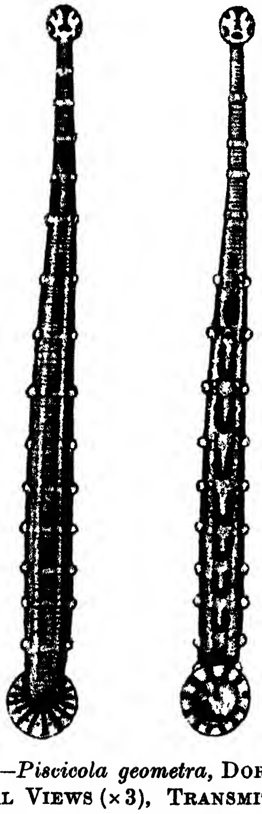


FIG. 245.—*Piscicola geometra*, DORSAL AND VENTRAL VIEWS (×3), TRANSMITTER OF TRYPANOSOMES AND TRYPANOPLASMS OF FRESH WATER FISH. (AFTER HARDING, 1910.)

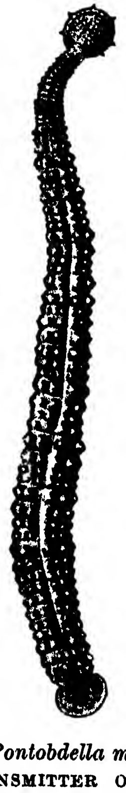


FIG. 246.—*Pontobdella muricata*, THE TRANSMITTER OF TRYPANOSOMES OF MARINE FISH (NATURAL SIZE). (AFTER HARDING, 1910.)

As long ago as 1857 Leydig had noted the presence of flagellates in the stomach of the leeches (*Piscicola* and *Pontobdella*) which had fed on fish, and Doflein (1901) suggested the possibility of these invertebrates being vectors of the fish trypanosomes. Keysselitz [cited by Hofer (1904)] was able to transmit the trypanosomes of tench, carp, and pike by means

of the leech, *Piscicola geometra* (Fig. 245). Brumpt (1904) observed the development of enormous numbers of trypanosomes in the stomach of *Hemiclepsis marginata* which had fed on infected fish, and Léger, L. (1904*e*), made a similar observation with species of *Piscicola* fed on loaches infected with *T. barbatulæ*. Brumpt (1905) succeeded in infecting young carp and two bull-heads by exposing them to the bites of leeches. Brumpt then traced the development of *T. granulosum* of the eel in *H. marginata*, and of various trypanosomes of marine fish—*T. solæ* and *T. cotti* in *Trachelobdella punctata*, and *T. scyllii* and *T. rajæ* in *Pontobdella muricata*. In the case of *T. granulosum*, he noted that after multiplication had taken place in the stomach of the leech the flagellates migrated forwards and passed through the proboscis into the proboscis sheath, whence infection of the wound inflicted by the proboscis took place. Neumann (1908, 1909) described the development of *T. giganteum* and *T. variabile* of the skate in the leech (*Pontobdella*), and was able to infect *Raja punctata* with *T. variabile* by means of *P. muricata* (Fig. 246).

Cycle in the Leech.—Robertson (1907) has studied the trypanosomes in *P. murciata*, and suggested the possibility of their being derived from the trypanosome (*T. rajæ*) of the skate (Fig. 247). A further contribution (1909, 1909*a*) to the subject was made by this observer, and the flagellates of the leech were definitely associated with *T. rajæ*. The first stages of development in the leech, according to Robertson, is a rounding-off of the trypanosome, with loss of undulating membrane and flagellum (Fig. 247, 1-10). The latter is finally cast off from the body, and may continue its movements in this free condition for some time. The rounded cytoplasmic body resulting from this change then undergoes division. The whole process can be watched under the microscope in a fresh blood-preparation. The single nucleus can be seen at the centre of the parasite, and its division into two can be followed. After division of the nucleus the cytoplasm divides, and two smaller bodies are produced. These in their turn divide, and the four daughter individuals repeat the process. In a film thirty-six hours after preparation there were present still unaltered trypanosomes actively motile and non-motile individuals in groups of four, six, or eight. At about this stage in the daughter forms there appear short stiff rods which by gradual growth become flagella. They seem to take about twelve hours or more to become motile. The flagellate forms thus produced are more or less rounded, and by change in shape and elongation, during which further multiplication occurs, various types of flagellate, some of which have the crithidia forms, arise (Fig. 247, 11). In the leech the rounding-off process and division into non-flagellate daughter forms and the early formation of the flagella take place in what Robertson calls the first stage of digestion. The production of the large number of

FIG. 247.—DEVELOPMENT OF *Trypanosoma rajæ* IN THE LEECH, *Pontobdella muricata* (2-14, ×4,500; 1, 15, 16, LOWER MAGNIFICATION). (AFTER ROBERTSON, 1907 AND 1909.)

[*For description see opposite page.*

flagellates of various types occurs during the middle period, when the blood is being digested into a green-brown fluid (Fig. 247, 12-14). In the third period of digestion the crop or stomach becomes nearly empty, and long, slender, very active flagellates of the typical trypanosome type appear. These forms migrate forwards, and presumably find their way into the proboscis sheath, though this is not actually mentioned (Fig. 247, 15-16). The whole developmental process is very similar to that of *T. vittatæ* of the tortoise described above. It is presumably the long narrow trypanosomes of the proboscis which bring about infection of the vertebrate.

The development of *T. granulosum* of the eel in *Hemiclepsis*, as described by Brumpt, is very similar to that of *T. rajæ*. At the end of twenty-four hours after feeding, however, flagellates had vanished from the stomach, and were undergoing development as leptomonas (? crithidia) forms in the intestine, whence they eventually migrated to the stomach and along the œsophagus to the proboscis and its sheath, where the metacyclic trypanosomes were to be found.

According to the observations of Brumpt (1904-1906), the trypanosomes of fresh-water fish are carried by *Hemiclepsis marginata*, in which the development is of two types (Fig. 240).

I. The trypanosomes develop in the stomach alone, and here the crithidia forms and eventually the metacyclic trypanosomes appear. There is no infection of the intestine nor of the proboscis sheath. Infection of the fish takes place by active migration or regurgitation forwards of the metacyclic trypanosomes while the leech feeds. To this category belong *T. abramidis*, *T. remaki*, *T. barbi*, *T. percæ*, *T. acerinæ*, and *T. squalii*.

II. The trypanosomes develop in the stomach and then pass into the intestine, where the flagellates persist. Before the leeches become infective the intestinal forms reinfect the stomach, from which the proboscis sheath is infected with metacyclic trypanosomes. To this group belong *T. granulosum*, *T. danilewskyi*, *T. phoxini*, and *T. carassii*.

In the case of other trypanosomes, only part of the cycle was observed. A development in the stomach was followed, but the subsequent events were not traced. To this group belong *T. barbatulæ*, *T. langeroni*, *T. scardinii*, *T. leucisci*, and *T. elegans*. Tanabe (1924) has noted that the trypanosome of the Japanese loach (*Misgurnus anguillicaudatus*) multiplies for a period of three or four days in the intestine of the leech (*Hirudo nipponica*). No transmission experiments were carried out.

1. Large form in blood of skate.
2-10. Rounded forms from the alimentary tract of the skate. Some of these are without flagella, and most of them are in process of division.
11. Crithidia form in crop of leech. 12-14. Trypanosome forms from crop of leech.
15-16. Slender forms from the proboscis of the leech.

As regards marine fish, Brumpt (1906) studied the development of *T. cotti* and *T. solæ* in *Trachelobdella punctata*. As was subsequently confirmed by Robertson (1909) in the case of *T. rajæ*, the trypanosomes lose their flagella, and active multiplication in the non-flagellate condition takes place. It is only after some days that crithidia and trypanosome forms reappear. The development is confined to the stomach. In the case of *T. scyllii* and *T. rajæ*, the same type of development occurs in

FIG. 248.—THE TRYPANOSOME OF THE GOLDFISH IN CULTURE (× *ca.* 2,000). (AFTER THOMSON, J. D., 1908.)

1-2. Forms from the blood of the fish. 3-5. Crithidia forms in early cultures.
6. Granular crithidia forms in older cultures.
7-8. Crithidia form and metacyclic trypanosome form from culture on the forty-third day.

Pontobdella muricata, but infection of the intestine follows the stomach phase, whereupon the forms in the stomach disappear. In no case did Brumpt observe infection of the proboscis sheath. In one instance, *T. cotti* was transmitted to a fish (*Cottus bubalis*) by the bite of an infected leech.

Culture.—The trypanosomes of fish are easily cultivated in blood-agar media. Thomson, J. D. (1908), cultivated the form in the goldfish, and

made the interesting observation that in old cultures there appeared trypanosome forms, which were evolved from the crithidia forms which occurred earlier. This was a clear demonstration that the cultural forms resemble in type and sequence those which occur in the invertebrate host (Fig. 248).

3. *Family:* BODONIDÆ Doflein, 1901.

In this family are included a number of flagellates which have one or more anteriorly directed flagella, and one which is often, though not always, longer and thicker than the others, and which trails behind the organism during progression as a trailing flagellum. The simplest forms belong to the genus *Bodo*, and have been described under various names (*Cystomonas* Blanchard, 1885, *Prowazekia* Hartmann and Chagas, 1910, etc.) as occurring in human fæces and also urine.

Genus: Bodo (Ehrenberg, 1830) Stein, 1875.

The flagellates belonging to this genus have ovoid bodies, an anterolateral cytostome, a central nucleus and a kinetoplast consisting of a parabasal body, and two blepharoplasts, from which arise the axonemes of the two flagella. Species of *Bodo* occur commonly in stagnant water and infusions, so their presence in fæces and urine is usually the result of the development of encysted forms which have gained entrance to the material from the air, or the receptacle, or have been ingested and passed through the alimentary canal. In the case of the fæcal forms, it seems clear that in all cases the organisms which have been described as *Bodo* or *Prowazekia* have been purely coprozoic forms which have developed after the stool has been passed. Thus Porter (1918) describes cases of human infection with *P. cruzi* and *B. stercoralis* in South Africa without producing any evidence that such extraneous sources of contamination have been excluded. A number of observers have claimed to have found *Bodo*-like organisms in urine. None of these accounts is entirely satisfactory, and having regard to the fact that organisms develop very rapidly in decomposing urine outside the body, in most cases it is safe to assume that the flagellates have developed after the urine had been passed. In other cases the flagellates may have been *Trichomonas*, which are known to occur in the urethra, and are quickly changed in appearance by the action of urine. In one instance, however, Powell and Kohiyar (1920) have described a case in which flagellates were present in the urine drawn aseptically from the bladder of a man in India. The case was repeatedly examined during five years, and the organism was constantly present. It is described as a " Bodo-like " organism, but the details of its structure were not accurately made out. The writer has examined some of the fixed material, and can only say that the flagellate which was present had

a round or ovoid body, a nucleus, and two flagella. It seems quite possible that it is actually a species of *Bodo*, but more than this cannot be stated.

Knowles and Das Gupta (1924) have seen a species of *Bodo* in the saliva from the human mouth. It was seen on three occasions between August 18 and October 27. Knowles, Napier, and Smith (1924) record the occurrence of a flagellate belonging to this genus in the rectum of the sand fly, *Phlebotomus minutus*, in India. To Alexeieff (1910*a*) is due the credit of first pointing out that the flagellates described as *Prowazekia* did not differ structurally from those of the genus *Bodo*.

Bodo caudatus (Dujardin, 1841).—The synonymy of this species is given by Dobell and O'Connor (1921) as follows: *Amphimonas caudata* Dujardin, 1841; *Bodo urinarius* Hassall, 1859; *Diplomastix caudata* Kent, 1881; *B. asiaticus* Castellani and Chalmers, 1910; *Prowazekia cruzi* Hartmann and Chagas, 1910; *P. weinbergi* Mathis and Leger, 1910; *P. asiatica* (Castellani and Chalmers) Whitmore, 1911; *P. javanensis* Flu, 1912; *P. urinaria* (Hassall) Sinton, 1912; *P. italica* Sangiorgi and Ugdulena, 1916; and with these must be included *B. stercoralis* Porter, 1918, and *P. ninæ kohl-yakimovi* Yakimoff, 1916.

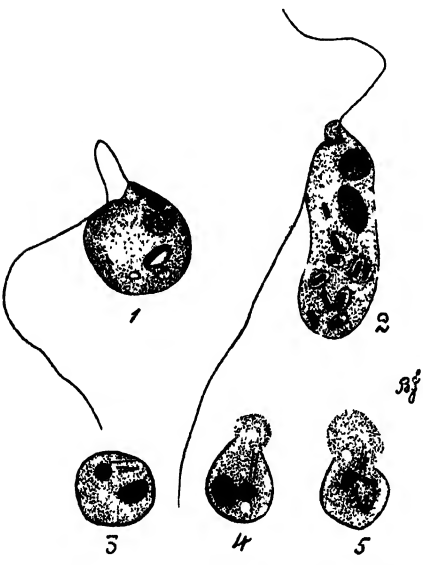


FIG. 249.—*Bodo caudatus* FROM HUMAN URINE (× 1,650). (AFTER SINTON, 1912.)

1-2. Two types of flagellate.
3. Encysted form.
4-5. Emergence of flagellate from cyst.

It is probable that *B. caudatus* is the commonest coprozoic flagellate to appear in human fæces. It is in no sense an inhabitant of the human intestine, but develops in fæces after they have left the body, and can often be obtained by inoculating fæces on to agar plates.

The forms which develop in decomposing urine, as described by Hassall (1859) and Sinton (1912), are probably the same species (Fig. 249). According to Klebs (1892), the flagellates vary in length from 11 to 19 microns, but the shape of the body varies. It may be long and slender or more or less rounded. The posterior end of the body is pointed and the body is somewhat flattened. At one side of the anterior extremity is the mouth, which may be considered to be on the ventral surface. Dorsal to the mouth is a small contractile vacuole, near which is the kinetoplast, a structure made up of a deeply staining parabasal and two blepharoplasts, each of which gives origin to an axoneme which passes to the surface of the anterior end of the body to form a flagellum. There is a

nucleus at the centre of the body consisting of nuclear membrane and large central karyosome. The cytoplasm contains various food vacuoles, especially in the posterior region. The two flagella which arise from the anterior end of the body are of unequal length. One is anteriorly directed, and is about the same length as the body, while the posteriorly directed one, which trails over the body in progression, is about twice this length.

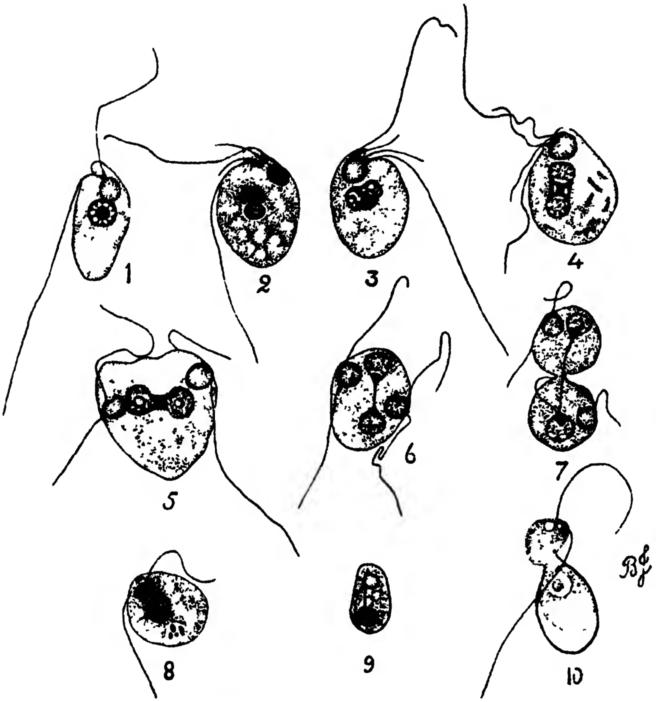


FIG. 250.—*Bodo edax* (× 1,400). (AFTER KÜHN, 1915)

1. Flagellate showing nucleus, kinetoplast, large contractile vacuole, and two flagella.
2. Division of blepharoplasts and formation of two new flagella; separation of nucleus into two parts.
3. Commencing division of the nucleus, the karyosome occupying the hollow of the dumb-bell-shaped structure.
4. One part of the nucleus divided, with karyosome in the middle.
5. More advanced stage with two kinetoplasts, each with two flagella.
6. Nucleus elongated and karyosome now dividing.
7. Division of body and long drawn-out karyosome.
8. One of products of division, nucleus being reconstructed.
9. Encysted form. 10. Escape of flagellate from cyst.

Dobell and O'Connor (1921) state that the trailing flagellum may be attached to the surface of the body for a short distance. The cysts of the organism are ovoid bodies 5 to 7 microns in length. As a rule, each cyst contains a single nucleus and kinetoplast, though in some cases, as a result of division, two of each of these may be present.

The flagellate multiplies by longitudinal fission after division of the kinetoplast and nucleus. The organism is readily cultivated in hay and other infusions, and it will also multiply on the surface of agar plates. According to Sinton (1912), division of the flagellate takes place once in every four hours, so that in a short time very large numbers are present in the medium.

Bodo edax Klebs, 1892.—This is another species which may appear in fæces, though less commonly than *B. caudatus* (Fig. 250). It is slightly smaller than *B. caudatus*, and more stumpy in form. The two flagella are

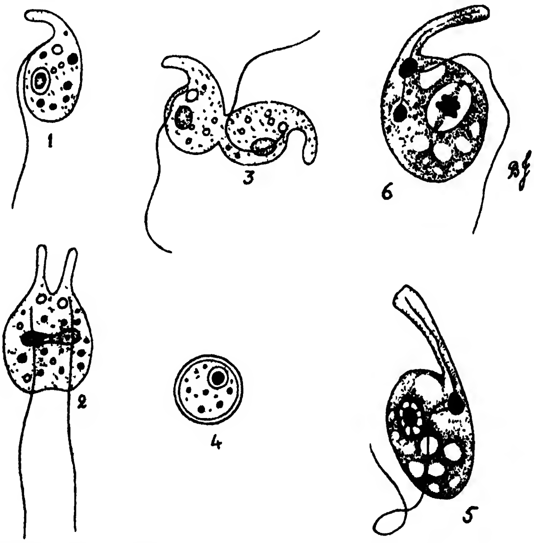


FIG. 251.—*Rhynchomonas nasuta* (1-4, × 1,800; 5-6, × 3,800). (1-4, AFTER PARISI, 1910; 5-6, AFTER BĚLAŘ, 1915.)

1. Usual type of flagellate. 2-3. Dividing forms. 4. Encysted form.
5. Stained flagellate, showing details of structure.
6. Dividing form with two kinetoplasts and nucleus dividing by mitosis.

approximately equal in length, and both are longer than the body. The organism was studied by Kühn (1915). In its method of multiplication and cyst formation it is very similar to *B. caudatus*.

Genus: Rhynchomonas Klebs, 1892.

Stokes (1888) in America described as *Heteromita nasuta* a flagellate which was ovoid in shape and provided with one trailing flagellum, over the point of origin of which there extended a digital process. Klebs (1892) created the genus *Rhynchomonas* for this organism. It was seen as a free-living flagellate in fresh water by Stokes (1888) and Bělař (1915), and in salt water by Griessmann (1913), while Parisi (1910) obtained it as a

coprozoic flagellate from the intestinal contents of cockroaches. Bělař, who obtained a culture of *Rhynchomonas nasuta,* has studied its structure and method of division (Fig. 251). The organism is ovoid in shape, and resembles members of the genus *Bodo* in the possession of a nucleus and kinetoplast. From the latter there arise two axonemes, one of which becomes a flagellum in the notch formed by the digital process, while the other is continued to the end of the process, but does not become a flagellum. The single flagellum acts as a trailing flagellum. The flagellate is evidently closely allied to species of *Bodo,* and the notch behind the digital process bears a striking resemblance to the cytostome of the flagellates of this genus. Not infrequently in such a form as *B. caudatus* the portion of the body in front of the cytostome has the appearance of a digital process, so that it does not seem improbable that the notch formed by the digital process may be actually a cytostome.

4. *Family:* PROWAZEKELLIDÆ Doflein, 1916.

This family includes the single genus *Prowazekella,* established by Alexeieff (1912), the members of which are parasitic in the intestine of lizards. The encysted forms are remarkable in that great increase in size takes place after the cyst wall has been formed.

Prowazekella lacertæ (Grassi, 1879).—Grassi (1879*a*), who first saw this flagellate, included it in his genus, *Monocercomonas.* He afterwards (1881*a*) placed it in Dujardin's genus, *Heteromita,* while Prowazek (1904*a*) referred it to the genus *Bodo.* Alexeieff (1911) described another form from the intestine of newts, salamanders, and axolotls, and included the two forms in the genus *Heteromita.* In the following year (1912*b*) he created the new genus, *Prowazekella,* for these flagellates.

P. lacertæ occurs in the intestine of lizards (*Lacerta, Tarentola,* etc.). The fully-grown flagellate has an elongate pyriform body, 10 to 30 microns in length (Fig. 252). It has a tapering posterior end and blunter anterior end, from which arise two flagella. One of these is directed forwards, and may be four times the length of the body, while the other is a trailing flagellum about twice the length of the body. The latter, in its backward course, is sometimes attached to the surface of the body for a short distance before becoming free. There is a nucleus near the anterior end, consisting of nuclear membrane and a central karyosome. Surrounding the nucleus are one or more bodies, the parabasals, while extending from the anterior end of the nuclear membrane is an axoneme (rhizoplast), which is continued into the two flagella. The life-cycle of the flagellate was described by Chatton (1917*b*) in the case of the form which occurs in the gecko, *Tarentola mauritanica.* The flagellate multiplies by longitudinal division in the gut of the lizard. Certain forms then lose their flagella, and, becoming ovoid in shape, produce cysts which have a diameter of

about 10 microns (Fig. 253). The writer (1921) has produced some evidence that two of the ovoid forms become encysted together, and that syngamy, with complete union of the cytoplasm and nuclei, follows (Fig. 254, *n-s*). A vacuole now appears in the cytoplasm, and the single nucleus begins to divide. The vacuole increases in size till the cytoplasm

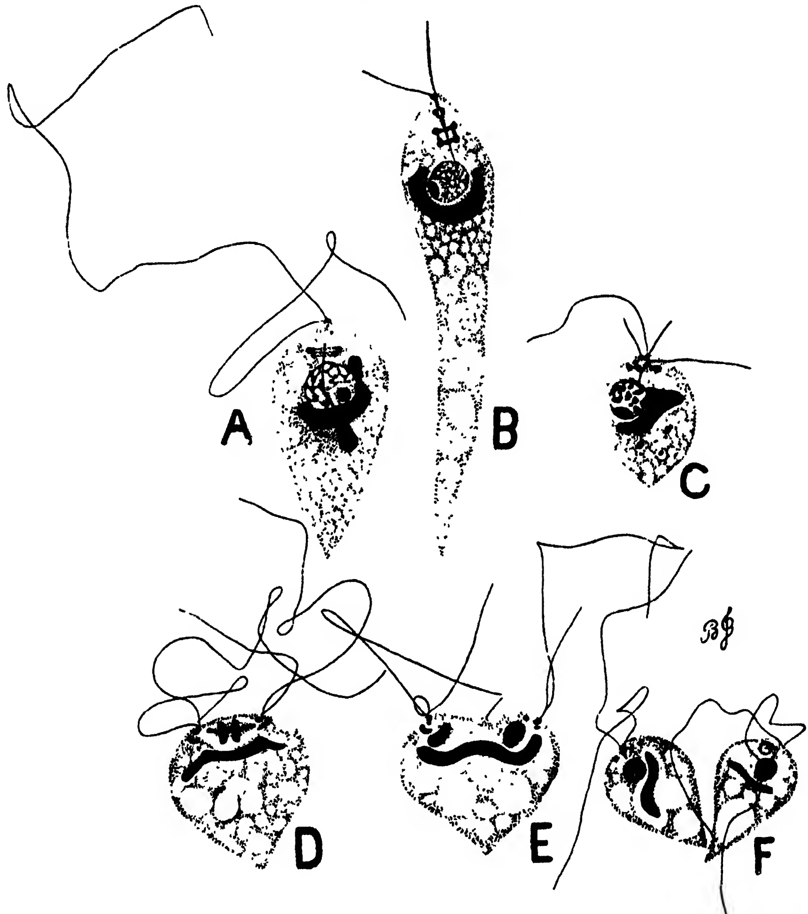


FIG. 252.—*Prowazekella lacertæ* (× 2,300). (AFTER BĚLAŘ, 1921.)

A-B. Two types of flagellate, showing nucleus, parabasal body, and flagellar connections. C-F. Stages in division.

is reduced to a thin layer lining the cyst. At the two-nuclear stage the nuclei lie at opposite poles of the cyst, which has a large central vacuole and bears a close resemblance to *Blastocystis*, with which it has been compared. Repeated divisions of the nuclei take place, while the cyst increases in size till it may reach a diameter of about 70 microns. At this

stage there are about sixty-four nuclei present. The cytoplasm still lines the cyst, which has a large central vacuole often traversed by thin strands of cytoplasm. According to Chatton (1917*b*), the cytoplasm then becomes heaped up round each nucleus, flagella are developed, and finally a number of small flagellates having the structure of the free forms are produced (Fig. 253). The cysts are found in large numbers in the hind-

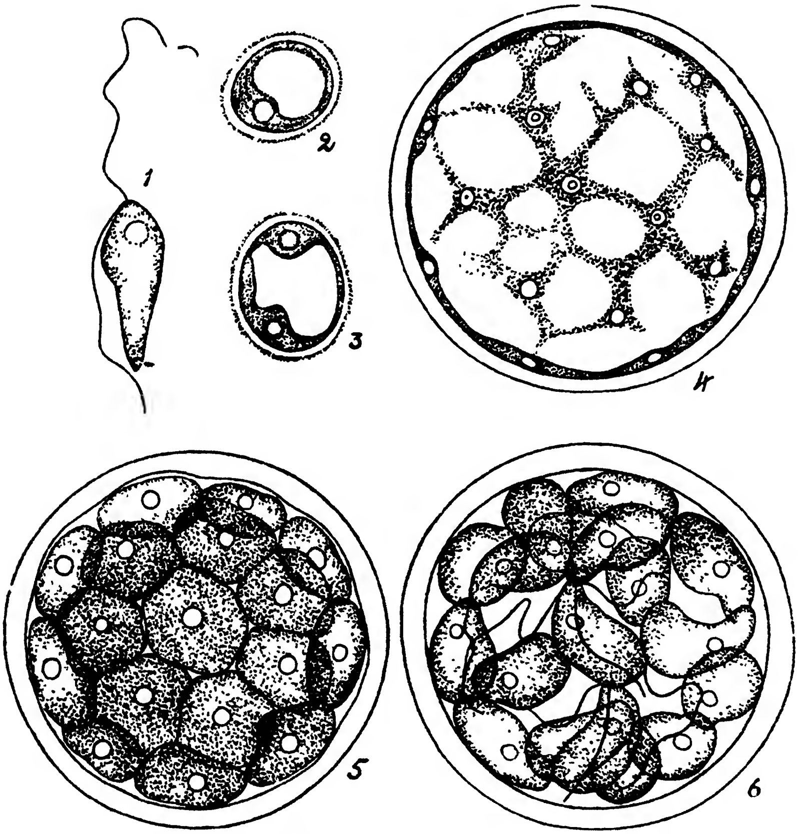


FIG. 253.—*Prowazekella lacertæ* FROM THE INTESTINE OF THE NORTH AFRICAN GECKO, *Tarentola mauritanica*, AS SEEN IN LIVING CONDITION (× 720). (AFTER CHATTON, 1917.)

1. Free flagellate. 2. Uninucleated cyst.
3-5. Growth of cyst, multiplication of nuclei and segmentation of contents.
6. Cyst containing flagellates.

gut of lizards, and they are passed in the fæces. Presumably, when the cysts are eaten by other lizards, the flagellates are liberated in the intestine.

The minute structure of the flagellate stage of *P. lacertæ* has been described by Bělař (1921*a*). The flagella actually rise from two minute granules, which are at the extreme anterior end of the flagellate

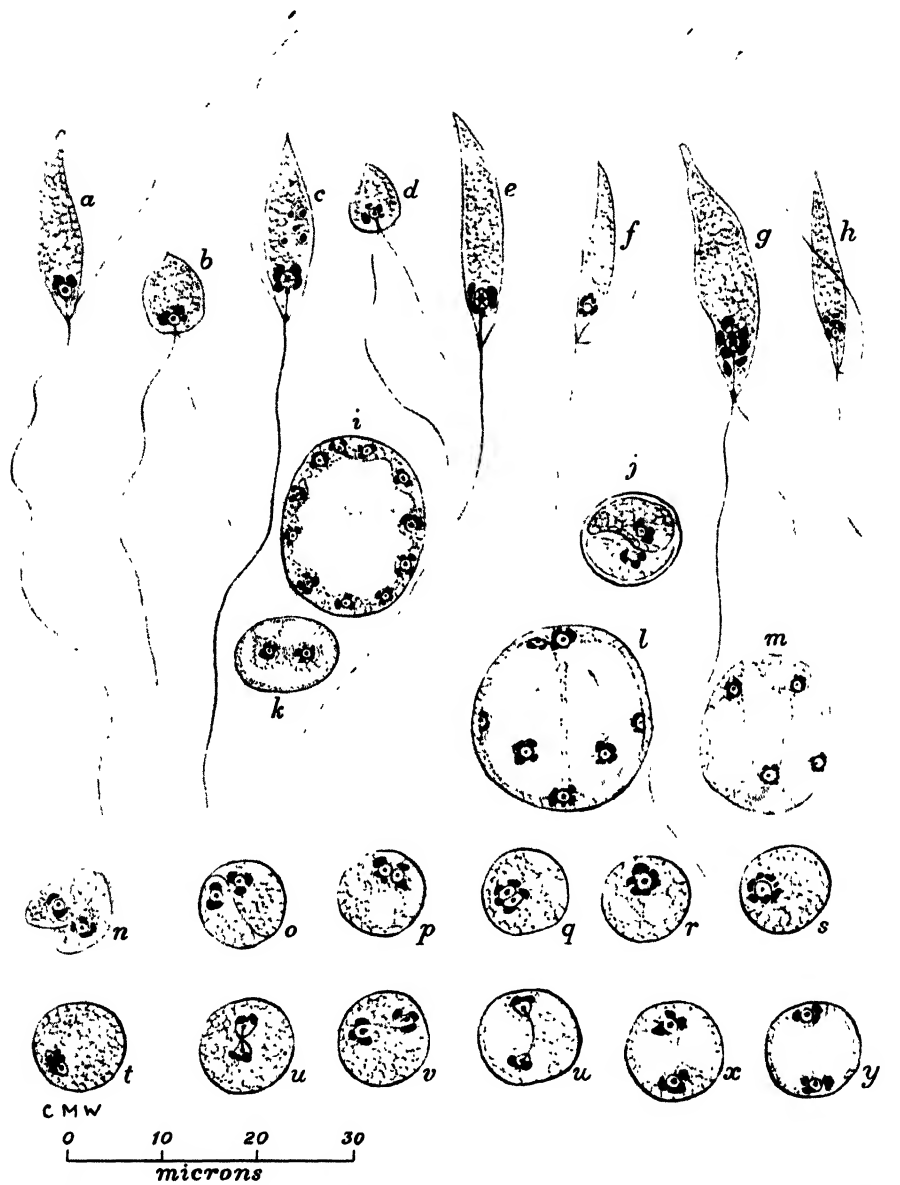


FIG. 254.—*Prowazekella lacertæ* FROM THE INTESTINE OF THE LIZARD (*Lacerta agilis*) (× 1,250). (AFTER WENYON, 1921; FROM *Parasitology*, vol. xii.)

a-h. Various types of flagellate. In some the backwardly directed flagellum is attached to the surface of the body for a short distance.
n-s. Probable stages in syngamy and encystment.
t-y. First nuclear division in zygote and formation of vacuole.
m, l, i. Growth of cyst and multiplication of nuclei.
j, k. Stages corresponding with those at *o* and *p*.

(Fig. 252). From each of these there passes backwards a rhizoplast (axoneme). The two soon merge into one another, and are continued to the nuclear membrane as a single rhizoplast. In addition to these structures, there are two rings. One surrounds the rhizoplast a short distance behind the basal granules or blepharoplasts, and a kind of funnel connects the ring with the rhizoplast. About half-way between the blepharoplasts and the nucleus is a second ring, which surrounds the rhiziplast. As already described, the spherical nucleus is surrounded by several bodies or a single elongated body which stains deeply. These may be regarded as parabasals. When the flagellate divides there is, first of all, division of the blepharoplasts, and two new flagella are formed. The two pairs of blepharoplasts then separate till they occupy the poles of the elongating nucleus. The chromatin becomes arranged at the equator of an intranuclear spindle in a compact mass formed by a group of chromosomes. The chromatin mass splits into two daughter plates, which pass to the poles of the nucleus which now divides. The parabasal body or bodies become arranged outside the nucleus as an elongate mass parallel to the nuclear spindle, and with nuclear division this divides into two parts. Finally, division of the flagellate takes place. The parabasal bodies persist in the cysts also, and at each division of a nucleus they are divided into two groups, one of which passes to each daughter nucleus. Prowazek (1904*a*) claimed to have demonstrated a process of autogamy within the cyst, but there is no evidence that such a process takes place. He concluded also that the cysts of the flagellate were identical with *Blastocystis*. This also is not correct. Typical *Blastocystis*, which is a vegetable organism, occurs in the intestine of lizards, and is easily mistaken for the cyst of *P. lacertæ* (Fig. 118).

Alexeieff (1911) discovered a form in the intestine of newts, salamanders, and axolotls. He regarded it as a distinct species, and later (1912*b*) gave it the name *P. longifilis*.

5. *Family:* EMBADOMONADIDÆ Alexeieff, 1917.

The flagellates belonging to this family have ovoid bodies and an anterior nucleus. On one side of the anterior end of the body is a cytostome, and anterior to it, and close to or actually upon the nuclear membrane, are two granules, the blepharoplasts, which give rise to two flagella. One flagellum is long and thin, and passes forwards as an anteriorly directed flagellum. The other flagellum is shorter and thicker, and passes backwards to protrude through the cytostome. There is a single genus which has the characters of the family.

Genus: **Embadomonas** Mackinnon, 1911.

The genus *Embadomonas* was founded by Mackinnon (1911, 1915) for flagellates found in the intestine of tipulid and trichopteran larvæ. Two species were described in these insects. Later a form was discovered by the writer and O'Connor (1917) in Egypt in the human intestine, since when other forms have been found in the intestine of vertebrates and invertebrates.

EMBADOMONAS IN MAN.

Embadomonas intestinalis (Wenyon and O'Connor, 1917).—This flagellate was found in man in Egypt in two cases by the writer and O'Connor (1917). They placed the flagellate in a new genus as *Waskia intestinalis*, but it was evident, as first pointed out by Chalmers and Pekkola (1918), that it really belongs to Mackinnon's genus *Embadomonas*. Fonseca (1920) expressed the opinion that the genus *Waskia* should be retained, but it is quite clear that the human parasite shows no features of generic value which will differentiate it from the genus *Embadomonas*. Since *E. intestinalis* was first described in Egypt, it has been discovered in other localities. It was found by Kofoid, Kornhauser, and Plate (1919) in New York in four men who had returned from overseas, and in four who had not been abroad. Hogue (1921*b*) has reported one case from Baltimore. Broughton-Alcock and Thomson (1922*a*) have seen a case in a man who had returned to London from China, while Jepps (1923) reports cases from Malaya. As will be shown below (p. 633), Chalmers and Pekkola (1919*a*) included this flagellate in their *Diplocercomonas sudanensis* which they described in the Sudan. A form identical in every way with *E. intestinalis* was seen by the writer in the cæcum of a guinea-pig which had been sent to Macedonia from Egypt in 1918, while he has cultivated the flagellate on three occasions from guinea-pigs, and once from a wild rat in England.

In the cases examined by the writer and O'Connor in Egypt the flagellates, when present in the diarrhœic stool, occurred in large numbers. In fresh material these were very active, and progressed in a peculiar jerky manner. The long thin anterior flagellum performed lashing movements, and it was evidently the organ of progression. The shorter and thicker flagellum which protruded through the cytostome had a more regular and slower action. The shape of the body varied considerably (Fig. 255). Some forms were elongate and about three times as long as they were broad, while others were almost spherical. Sometimes the posterior end of the body was drawn out into a tapering process. When seen with the cytostome at the side, the narrow forms often had an outline resembling that of a bird. In length the flagellates varied from 4 to 9 microns, and

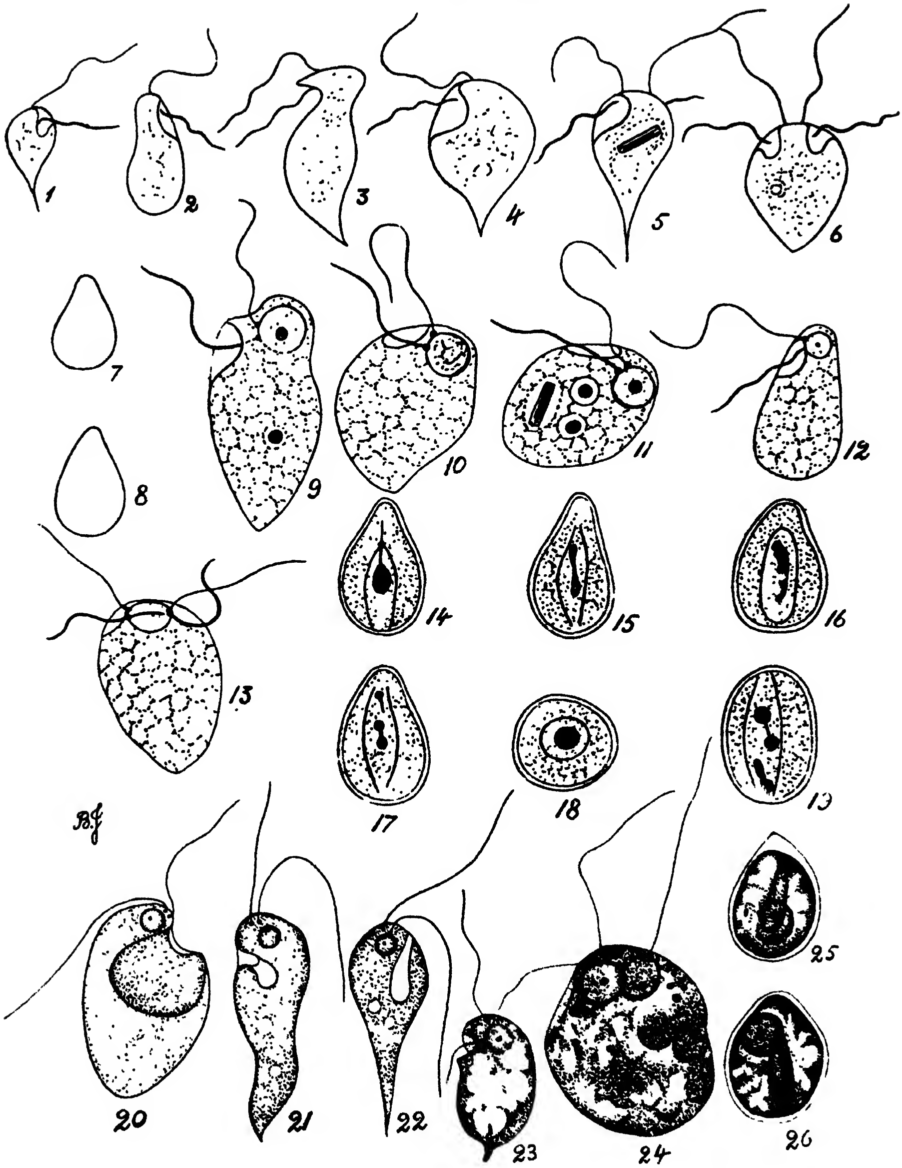


FIG. 255.—*Embadomonas intestinalis* FROM THE HUMAN INTESTINE. (AFTER WENYON AND O'CONNOR, 1917; FAUST, 1922; AND JEPPS, 1923.)

1-6. Appearance of flagellates in living condition (× *ca.* 3,000).
5-6. Dividing forms. 7-8. Cysts in fresh condition (× *ca.* 3,000)
9-12. Flagellates fixed and stained, showing relation of two blepharoplasts (× *ca.* 3,500).
13. Dividing form (× *ca.* 3,500). 14-17. Encysted forms stained (× *ca.* 3,500).
18. Cyst as seen from end (× *ca.* 3,500). 19. Ovoid cyst (× *ca.* 3,500).
20-22. Large forms described as *Embadomonas sinensis* by Faust (× *ca.* 2,000).
23-26. Free and encysted forms as depicted by Jepps (× *ca.* 3,500).

in breadth from 3 to 4 microns, while the spherical forms were about 9 microns in diameter. The anterior flagellum was as long or longer than the body, while the thicker cytostomal flagellum was shorter than this. Many of the spherical forms were evidently dividing flagellates, as they were seen to possess two cytostomes, one on each side of the anterior end of the body, and two pairs of flagella.

The encysted forms as seen in fresh material are whitish, opalescent, pear-shaped bodies (Fig. 255, 7-8). The anterior end is distinctly narrowed, and often forms a sort of tubercle. In the living condition they vary in length from 4·5 to 6 or even 7 microns, while the breadth varies from 3 to 4·5 microns. Dobell and O'Connor (1921) have given the dimensions of the cysts as less than this, but their measurements were taken from fixed and stained preparations made by the writer and O'Connor in Egypt. The writer has recently examined further fresh material, and can verify the measurements previously given in the account by the writer and O'Connor (1917). In stained films the flagellates are seen to have an alveolar cytoplasm, within which bacteria may occur in food vacuoles (Fig. 255, 9-13). Near the anterior end is the spherical nucleus, which has a central karyosome. On the nuclear membrane occur two granules, the blepharoplasts, from which the flagella arise. Several stages in the division process were seen, but the details were not followed. The spherical forms with two cytostomes were seen to have two nuclei and four flagella, while other similar forms were seen with an elongated dividing nucleus with a pair of blepharoplasts and two flagella at each extremity of the nucleus. It is possible that the margins of the cytostome are supported by marginal fibres, as in *Chilomastix*, but the small size of the organism makes it difficult to determine this point with accuracy.

In stained films the cysts are seen to have a somewhat peculiar internal structure. There are generally two dark lines marking out an elongate, oval, or spindle-shaped area within the cyst (Fig. 255, 14-19). It is often nearly as long as the cyst itself, and within it is what appears to be the karyosome of the nucleus. In some cysts two dumb-bell-shaped bodies or karyosomes are seen. The writer and O'Connor (1917) interpreted the structure as being a much elongated nucleus. A very similar body was figured by Dobell (1909), and interpreted as the elongated nucleus in cysts which he identified as being the encysted forms of *Trichomonas* of the frog, but which are probably cysts of a species of *Embadomonas* which the writer has seen in English frogs. Dobell and O'Connor (1921) figure the cyst of *E. intestinalis* as having a round, more or less central nucleus, and the outline of the cytostome at one side towards the anterior end. Jepps (1923) has figured the cyst as having an elongate central cytostome and a round nucleus with central karyosome (Fig. 255, 25-26). The cyst thus

resembles those of *Chilomastix mesnili*, but is smaller. Quite recently the writer has examined cysts of *E. intestinalis*, which have been fixed in osmic acid vapour and stained by Leishman stain. In these preparations there is a central red granule surrounded by a blue ring, which undoubtedly represents the nucleus and its karyosome. The two lines which extend the whole length of the cyst probably represent the margins of the cytostome, and not the nuclear membrane. The dumb-bell-shaped structures mentioned above may have been dividing karyosomes.

In one of the two infections seen by the writer and O'Connor (1917) in Egypt, *E. intestinalis* persisted for one and a half months. Though the flagellates were seen in diarrhœic conditions, there was no evidence that they were the actual cause of the trouble.

Hogue (1921*b*) reports the successful culture of *E. intestinalis* in a medium made by cooking the white of one egg with 100 c.c. of 0·7 per cent. solution of sodium chloride. During heating, the mixture is constantly shaken. It is then filtered and placed in test-tubes, after which it is autoclaved. By subculture every other day, the flagellate was kept alive for over eight weeks at a temperature of 35° C. In the cultures the flagellates multiply by binary fission, and also produce the typical cysts. The writer (1921*a*) has succeeded in cultivating *E. intestinalis* as also forms from the guinea-pig, rat, tortoise, and frog, not only in Hogue's egg medium, but also in a soft rabbit blood-agar medium. The cultures were maintained both at 24° and 30° C.

Faust and Wassell (1921) described as *E. sinensis* a flagellate seen by them in nine cases of diarrhœa in North China. A further account of the organism has been published by Faust (1922). The average size is given as 14 by 4·2 microns, but longer forms up to 20 microns were seen. From the description and figures it appears that the two flagella are of equal length and thickness (Fig. 255, 20-22). The encysted forms, however, correspond in shape and size with those of *E. intestinalis*. In a case of *E. intestinalis* (that of Broughton-Alcock and Thomson noted above) which the writer had an opportunity of studying, flagellates up to 17 microns in length were seen. The larger forms were more frequently encountered in cultures. The statement made by Faust and Wassell that the flagella of their species were equal in length and thickness requires confirmation, for in all the forms examined by the writer (man, guinea-pig, rat, tortoise, frog) the cytostomal flagellum has been thicker and shorter than the other. It seems to the writer that it is exceedingly doubtful if *E. sinensis* is a distinct species from *E. intestinalis*, especially as the encysted forms are alike. The flagellate described as *Enteromonas Bengalensis* by Chaterjee (1919) may be *E. intestinalis* (see p. 307).

EMBADOMONAS IN ANIMALS.

Embadomonas wenyoni (Fonseca, 1917).—This form closely resembles *E. intestinalis* of man, with which it may be identical. It was described by Fonseca (1917) as *Waskia wenyoni*, and was found in the cæcum of the Brazilian monkey, *Cebus carya.* The description referred to the spherical dividing forms, which have two sets of flagella and two cytostomes. They correspond in every way with the dividing stages of *E. intestinalis.*

E. agilis Mackinnon, 1911.—This flagellate was discovered by Mackinnon (1911, 1915) in tipulid and trichopteran larvæ. It varies in size from 4 to 1·5 microns to 11 by 3 microns. The cysts measure 3·5 to 4 by 4 by 3 microns.

E. alexeieffi Mackinnon, 1911.—This form, which is slightly larger than the preceding one, occurred only in tipulid larvæ. It measured 7 to 16 by 5 to 9 microns, while the cysts measured 5 to 6 by 4 to 5 microns. The cysts of this and *E. agilis* are described and figured as being ovoid in shape, with no tendency to a narrowing of the anterior end, as not infrequently occurs in those of *E. intestinalis.*

E. belostomæ (Brug, 1922).—Brug (1922) records as *Waskia belostomæa* an *Embadomonas* which he found in the water bug, *Belostoma* sp., in Java. It actually shows no specific differences from other species which have been described.

A flagellate of the genus *Embadomonas* has recently been seen by the writer in the intestine of a tortoise (*Testudo argentina*) which died in the Zoological Gardens in London (Fig. 11). Structurally, it did not differ from *E. intestinalis*, but was distinctly larger, as it varied in length from 12 to 19 microns. Encysted forms were not seen. It was successfully cultivated. A form having the same dimensions was discovered by the writer in the rectum of an English frog. A culture was obtained, and in this the characteristic cysts resembling those of *E. intestinalis* were produced.

6. *Family:* CHILOMASTIGIDÆ.

This family includes flagellates which have three anteriorly directed flagella, and one posteriorly directed one which lies in a long cytostomal cleft. Characteristic oval or pear-shaped cysts are produced, within which the single nucleus and the cytostomal cleft can be distinguished. It includes the single genus *Chilomastix.* The genus *Tetrachilomastix*, which was founded by Fonseca (1916) for flagellates having the *Chilomastix* structure, except the possession of four anterior flagella instead of three, is not free from doubt.

Genus: Chilomastix Alexeieff, 1910.

Alexeieff (1909) described as *Macrostoma caulleryi* a flagellate of this type from the intestine of tadpoles, and it was in this genus that the writer (1910*b*) placed the human form as *M. mesnili*. It was later discovered that the name *Macrostoma* was not available, so Alexeieff (1910) included the flagellate in Perty's genus *Tetramitus*. It was evident, however, that the parasitic forms were not of the same type as the free-living *Tetramitus*, so Alexeieff (1912*b*) established the new genus, *Chilomastix*, by which name these forms are now generally known.

The flagellates of this genus have pear-shaped bodies, three anteriorly directed flagella, and a large cytostomal cleft, within which is a fourth flagellum. There is a vesicular nucleus near the anterior end of the body, and between it and the anterior end of the cytostomal cleft is a group of blepharoplasts which give origin to the four flagella and to two filaments which pass along the margins or lips of the cytostomal cleft. Reproduction is by longitudinal division. Characteristic pear-shaped cysts are produced. In each cyst there is a single flagellate, of which the nucleus, cytostomal cleft, and blepharoplasts can often be clearly distinguished.

CHILOMASTIX IN MAN.

Chilomastix mesnili (Wenyon, 1910).—As pointed out by Brumpt (1912*a*) and Chalmers and Pekkola (1917), Davaine (1854) was the first observer to mention this flagellate. In 1860 he redescribed and figured it. Though his figures were imperfect in that only a single anterior flagellum was shown, his statements regarding the cytostomal cleft render it very probable that he was actually dealing with this organism. He referred to it as *Cercomonas hominis* variety A, the variety B being *Trichomonas*. In the same year Moquin-Tandon (1860), some months before the appearance of Davaine's work, referred to the latter's two varieties of *Cercomonas*, the account of which had not then been published. He must have had some knowledge of Davaine's forthcoming work, for, though he did not give any recognizable description or figures of the flagellates, for the variety "A" he proposed the name *Cercomonas davainei*, and for the variety "B" the name *Cercomonas obliqua*. It seems clear that if there is no doubt as to the identity of Davaine's flagellates, the correct name for the human *Chilomastix*, as pointed out by Kofoid (1920), should be *Chilomastix davainei* Moquin-Tandon, 1860, while that of the human intestinal *Trichomonas* should be *Trichomonas obliqua* Moquin-Tandon, 1860. It seems undesirable, however, to change the name *Chilomastix mesnili*, which is now in general use, and though it is very probable that Davaine was actually observing this flagellate, his description would have been quite inadequate to establish its identity were it not for the fact that the human intestine harbours only a limited number of organisms of distinctive structure. Davaine's description might apply to *Embadomonas intestinalis*, which, however, is a much rarer organism than *Chilomastix mesnili*. Cunningham (1871) in India, Marchand (1875), Leuckart (1879), Grassi (1881*a*), Epstein (1893), Roos (1893), and others probably saw this flagellate, but they did not describe it accurately, and confused it with *Trichomonas*. It must have been frequently referred to as *Cercomonas*, a name which was formerly employed by medical writers as a general name for any flagellate of the human intestine.

The flagellate named by Prowazek (1911) *Fanapepea intestinalis*, that by Prowazek and Werner (1914) *Cyathomastix hominis*, and that by Gäbel (1914) *Difämus*

tunensis are almost certainly identical with *Chilomastix mesnili*, though Gäbel failed to recognize the flagellum within the cytostome. The flagellates belonging to the genus *Enteromonas* established by Fonseca (1915) are probably, in some cases at least, small rounded forms of *Chilomastix* (see p. 307).

C. mesnili is usually about 10 to 15 microns in length, though very small spherical forms not more than 3 to 4 microns in diameter may be met with as well as larger ones up to 20 microns in length (Fig. 256). The anterior end is rounded or sometimes definitely flattened, while the posterior end varies considerably. It is sometimes blunt, and at other times drawn out into a long thin tapering tail. There is a long cytostomal cleft about half as long as the body itself, and this is obliquely arranged in such a way that, if a flagellate is observed with the cytostomal cleft upwards, the rounded anterior end pointing away from the observer and the posterior end towards him, then the anterior end of the cytostomal cleft is nearer the left side of the body of the flagellate, while the posterior end is nearer the right side. The two margins of the cytostomal cleft often form definite lips, which may even overlap one another. There is also a groove on the body which varies in development in different individuals. If the flagellate be regarded as in the position indicated above, then the groove commences near the anterior end of the body to the left of the cytostomal cleft, and passes round the body in a spiral manner parallel to the cleft (Fig. 256, 9-10). It may terminate at the posterior end of the cytostomal cleft, but is often continued beyond it, and may make two complete turns of the body. On account of this spiral groove, many of the flagellates appear to have the posterior region of the body curiously twisted. In some infections the spiral groove is not evident. There is a spherical nucleus near the anterior end of the body, and just anterior to it is a group of blepharoplasts, which, according to Dobell (Dobell and O'Connor, 1921) are six in number. The cytostomal cleft commences just behind the group of blepharoplasts. Three of the blepharoplasts are in front of the others, and from each of these there arises an axoneme which passes to the anterior surface of the body, there to enter one of the three anteriorly directed flagella. Each flagellum is about as long as the body. The three posterior blepharoplasts give rise to three different structures. The central one gives rise to a flagellum, which is thicker than the anterior flagella, and which lies in the cytostomal cleft. Some observers, as, for instance, Boeck (1921*a*), believe that there is a membrane within the cytostomal cleft, and that the flagellum is attached to its margin. This is probably not correct, as sometimes the flagellum leaves the cytostomal cleft in which it usually lies. If the flagellate is observed in the position described above, it will be noted that of the three posterior blepharoplasts that on the left gives rise to a deeply staining fibre,

which runs along the left margin of the cytostomal cleft, round its posterior end, and up the right side for a short distance. From the right blepharoplast another fibre passes along the right margin of the cytostomal cleft for about three-quarters of its length to a point near which the other

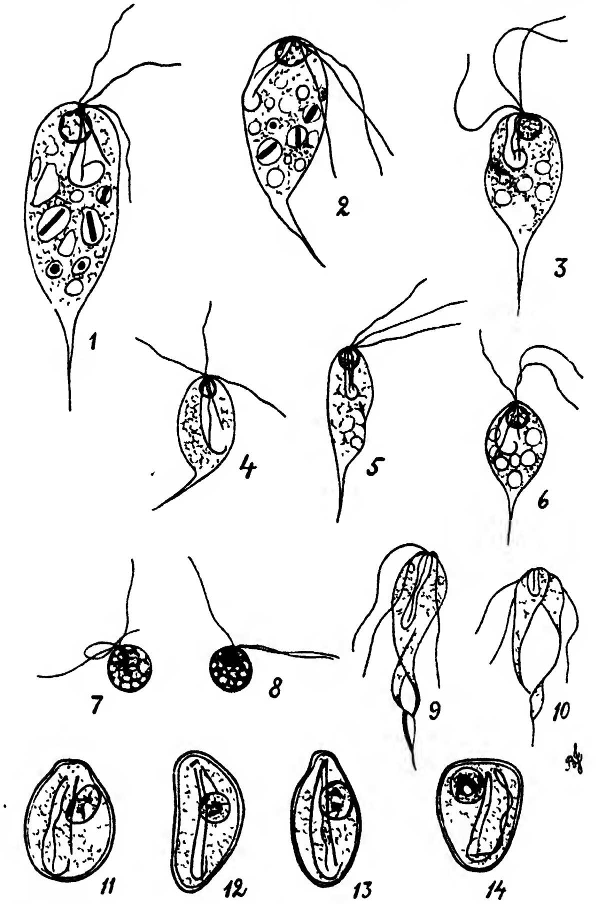


FIG. 256.—*Chilomastix mesnili* FROM THE HUMAN INTESTINE (× 2,000). (7-8, AFTER WENYON, 1914; 9-10, AFTER WENYON AND O'CONNOR, 1917.)

1-6. Ordinary forms as seen in stained films.
7-8. Small globular forms in which the cytostomal groove is not apparent.
9-10. Drawings of living specimens, showing the twisted appearance of the posterior region of the body. 11-14. Encysted forms in stained films.

marginal fibre terminates. The six blepharoplasts are usually so closely packed together that they appear as a single deeply staining body. The mouth or cytostome is at the posterior end of the cytostomal cleft at the point where the marginal fibre turns round the posterior margin of the cleft.

Kofoid and Swezy (1920) have given another interpretation of the structure of the cytostomal cleft and blepharoplasts (Fig. 69). According to them, the cytostome is an elongate aperture at the bottom of the cleft. It is described as having the shape of the outline of a dumb-bell and supported by a fibre running completely round its margin. This fibre, as also those found on the margins of the cleft itself, are said to originate from one of the blepharoplasts. Of the latter, the left-hand one, which turns round the posterior margin of the cleft, is called the *peristomal fibre* and the other one the *parabasal*. As regards the blepharoplasts, Kofoid and Swezy describe three which are united with one another by various fibres called rhizoplasts, and with a granule on the nuclear membrane which they call the centrosome. There seems little ground for homologizing one of the marginal fibres with the parabasal bodies of other flagellates, while the interpretation of the group of blepharoplasts is open to question, especially as Bělař (1921*a*) has published a description of the structure of *Chilomastix aulastomi* of the leech (*Aulastomum gulo*), which agrees entirely with the account given above as far as the blepharoplasts and cytostomal apparatus are concerned.

The method of multiplication of *C. mesnili* is undoubtedly by longitudinal fission after division of the nucleus. Though the writer has seen isolated stages of this process, it has not been followed in detail. The longitudinal fission of *C. aulastomi* has been described by Bělař (1921*a*), and it may be presumed that the division of *C. mesnili* will be very similar (Fig. 257). Apparently, the cytostomal cleft and its fibres vanish, and a single granule appears in place of the group of blepharoplasts, which may be supposed to have become more closely packed together. This granule is on the surface of the nuclear membrane, and it divides into two. The two granules then take up positions at opposite poles of the nucleus, and an intranuclear spindle is formed between them. The nucleus, which retains its membrane, then moves to a more central position, and the chromatin of the nucleus becomes arranged at the equator of the spindle in the form of a plate of chromosomes. Two daughter plates are formed by division of the chromosomes, and these move to opposite poles of the spindle. Meanwhile, new flagella begin to grow out from the two granules, which then subdivide into the several blepharoplasts. The elongated nucleus is finally divided at its centre, and the daughter nuclei assume the characters of the nucleus of the adult flagellate. Two new cytostomal clefts and the other structures associated with them are formed. The body of the flagellate now divides by constriction, and two flagellates result.

C. mesnili is often found in the stool in the encysted condition. The cysts, which were first described and figured by Prowazek and Werner

(1914), are pear-shaped structures which vary in length from 7 to 10 microns (Fig. 256, 11-14). In the majority of cysts one end is narrower than the other, though occasionally the two ends are more alike. In the fresh condition practically nothing of the internal structure can be made out,

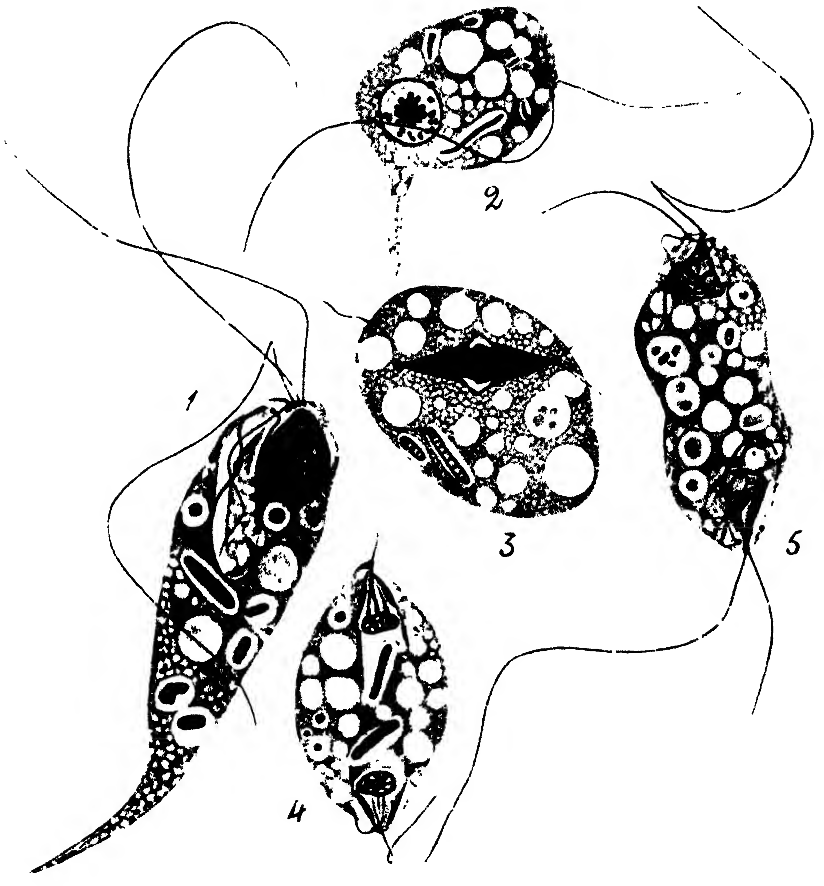


FIG. 257.—*Chilomastix aulastomi* (× 2,300). (AFTER BĚLAŘ, 1921.)

1. Usual type of flagellate.
2. Commencing division with two centrosomes on nuclear membrane.
3. Spindle with centrosomes and chromosomes at equator of spindle; new flagella are forming.
4. Nuclear division nearly complete.
5. Commencing division of flagellate

though a few greenish refractile granules are sometimes seen. In iodine solution the single round nucleus and the cytostomal cleft can be faintly distinguished (Plate II., 24, p. 250). In stained specimens, however, practically all the details which can be seen in the flagellates themselves are visible. The nucleus is near the narrow end of the cyst, and near it can

be seen the group of blepharoplasts, which are often more scattered than in the flagellates themselves.

The cytostomal cleft can be seen extending for the greater part of the length of the cyst, while the flagellum can often be detected within it. The cysts most usually remain in this condition, and are passed from the body sometimes in large numbers, so that several can be seen in every field of the twelfth objective. Though large numbers of the cysts have been examined by the writer and other observers, no indication of nuclear division has been noted. Kofoid and Swezy (1920), however, describe a division process within the cyst. Division of the blepharoplasts is followed by mitotic division of the nucleus, while the cytostomal cleft and the marginal fibres are duplicated. There results a cyst containing a flagellate

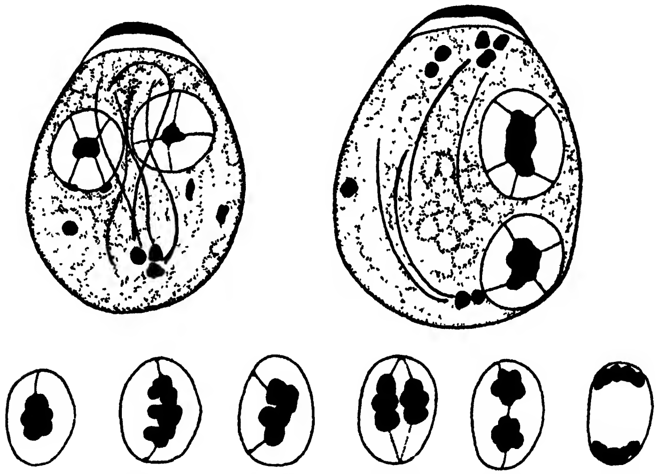


FIG. 258.—CYSTS OF *Chilomastix mesnili* WITH TWO NUCLEI: SIX NUCLEI IN VARIOUS PHASES OF MITOSIS (× 3,500). (AFTER HEGNER, 1923.)

with two sets of the various structures possessed by the ordinary cysts. Division of the cytoplasm into two flagellates would presumably be the next stage, but this was not observed. Hegner (1923*b*) has also observed binucleate cysts of *C. mesnili*, and has noted that the single nucleus divides by mitosis in which about five chromosomes are present (Fig. 258). Binucleate cysts are undoubtedly of rare occurrence, as no other observers have seen them.

C. mesnili is sometimes present in very large numbers in diarrhœic stools, both in the free and encysted condition. In formed stools only the cysts are found. The persistence of the infections is well illustrated by a case observed by the writer and O'Connor (1917) in Egypt, where *C. mesnili* was continually present during an observation period of fifty days. In another case it was present for ninety days, except for an interval of a

month, when it was apparently absent or present in such small numbers as to escape detection. As in other flagellate infections, the number of organisms present is subject to marked periodic fluctuations. The evidence as to pathogenicity is still wanting. The writer (1920) has noted that in sections of the large intestine the flagellates may be found within the lumen of the glands, but could find no indication that invasion of the tissues could take place. Kessel (1924*a*) reports the successful inoculation of monkeys with *C. mesnili*.

The culture of *C. mesnili* has been successfully carried out by Boeck (1921*a*), who used a medium consisting of one part of human serum to four parts of Locke's solution, to which 0·25 grain of dextrose had been added. In this medium at 37° C. the flagellates survived and multiplied for eight or nine days till they were overgrown by the bacteria. By subculture every two or three days the strain was maintained for about five months. Reichenow (1923) has also cultivated the organism in a medium prepared by dropping dilute serum into hot saline, so that flocculi are formed. He has grown it from stools which were microscopically negative, a fact which demonstrates the value of the culture method for diagnostic purposes. Boeck and Drbohlav (1925), and Thomson, J. G., and Robertson (1925), report the culture of *C. mesnili* in Boeck's L.E.S. medium.

The small round forms of *C. mesnili*, which have a diameter of 3 to 6 microns, often have the cytostomal groove obscured (Fig. 256, 7-8). In this condition they resemble the flagellate described as *Enteromonas hominis* by Fonseca. From an examination of their films, the writer is able to state that the cases of *E. hominis* infections recorded from the Sudan by Chalmers and Pekkola are in reality ones of *C. mesnili*, in which the majority of the flagellates are in the small rounded form. This fact raises the question as to whether the other cases of *E. hominis* infection are not due to the same organisms. If this be so, the name *Enteromonas* becomes a synonym of *Chilomastix* (see p. 307).

CHILOMASTIX IN ANIMALS.

Flagellates of this genus are fairly common parasites of animals. Though many of these have been given specific names, it is very doubtful if they can be distinguished from one another. *C. mesnili* varies so much in size, as also do the cysts, that this feature is of little value in the differentiation of species. Thus Alexeieff (1914) expressed his belief that the human flagellate, *C. mesnili*, is identical with *C. caulleryi* of frogs.

C. bettencourti Fonseca, 1915, is parasitic in the intestine of rats (*Rattus norvegicus*). This form has been seen by the writer on several occasions in both rats and mice, and he can find no differences between it and the human form. Fantham (1925) records as *C. muris* a form in the gerbil (*Tatera*

lobengula) and the rat (*Rattus coucha*) of South Africa. *C. capræ* Fonseca, 1915, is a very similar form found in the rumen of goats (*Capra hircus*).

C. cuniculi Fonseca, 1915, occurs in the cæcum of rabbits (*Oryctolagus cuniculus*). The form named *C. cuniculi* var. *rossica* by Yakimoff, Wassilewsky, Korniloff, and Zwietkoff (1921) is unquestionably identical with *C. cuniculi*.

C. rosenbuschi Fonseca, 1916, occurs in the intestine of the viscacha (*Lagostomus maximus*), a South American rodent, and *C. intestinalis* Kuczynski (1914) in the guinea-pig (*Cavia porcellus*). The latter is fairly commonly present in guinea-pigs in England. Chalmers and Pekkola (1918) recorded the occurrence of a *Chilomastix* in the gerbil (*Gerbillus pygurthus*) of the Sudan. Bach (1923) has seen the cysts and free forms of a *Chilomastix* in a monkey, *Macacus rhesus*, and Hegner (1924) the cysts in another monkey, *Cebus apella*. Species of *Chilomastix* occur in other hosts than mammals. Thus, Alexeieff (1909) described *C. caulleryi* from the intestine of tadpoles, axolotls, and salamanders. A form, probably *C. caulleryi*, was seen by Fantham (1922) in the South African clawed toad (*Xenopus lævis*). Alexeieff (1910) also saw a flagellate of the same type in the marine fish, *Motella tricirrata* and *M. mustela*. He (1912*b*) gave it the name *C. motellæ*, while another form which he saw in the fish, *Box salpa*, he identified with the human *C. mesnili*. Brumpt (1912*a*), however, regarded it as a distinct species, and gave it the name *C. bocis*. Martin and Robertson (1911) mention the occurrence of a species of *Chilomastix* in the intestine of the coal fish (*Gadus virens*). The writer (1921) recorded *Chilomastix* sp., a small form from the gut of two Egyptian lizards, *Lacerta agilis* and *Agama stellio*, and he has since seen similar forms in films made by Chalmers and Pekkola of the intestinal contents of the gecko, *Tarentola annulurus* of the Sudan. Bělař (1921*a*) has described as *C. aulastomi* a species which occurs in the hind-gut of the horse leech, *Aulastomum gulo*. It is possibly this form which Alexeieff (1910) records as having been seen by Chatton in *Hæmopsis sanguisuga*. The writer has seen a flagellate, probably *C. caulleryi*, in the common English toad. Both free and encysted stages occurred.

Under the name of *C. gallinarum*, Martin and Robertson (1911) described a flagellate from the cæcum of fowls (Fig. 265, A). According to their description, there were four anterior flagella, but no mention is made of one within the cytostomal cleft. Fonseca (1916) created the genus *Tetrachilomastix* for flagellates of this type, and later (1920) states that there is a fifth flagellum within the cytostome. The chicken parasite would then become *T. gallinarum*, differing from species of *Chilomastix* in having four instead of three anterior flagella. The encysted stages are similar to those of species of *Chilomastix*. The writer has studied the

fowl flagellate, and finds that only four flagella are present, and that it has the characteristic structure of members of the genus *Chilomastix*. In the form figured by Martin and Robertson the cytostomal flagellum was evidently in an unusual position outside the cytostomal cleft. Fonseca's genus, *Tetrachilomastix*, thus becomes a synonym of *Chilomastix*. Sangiorgi (1917) placed in this genus as *T. intestinalis* a flagellate he saw in human fæces. It had four anterior flagella and was cultivated. There is no evidence that it was not a *Trichomonas*.

Chatterjee (1923) has given the name *Tetrachilomastix bengalensis* to a flagellate which he says occurs commonly in the human intestine in India. According to his description, it has the general structure of a *Chilomastix*, but differs in that there are four anterior flagella, while a fifth runs along the border of an undulating membrane situated at one side of the large cytostomal groove. The attached axoneme may extend posteriorly as a flagellum. Through the courtesy of the author the writer has been able to examine preparations of the flagellate, and he is quite unable to convince himself that the organism differs in any essential respect from *C. mesnili*. It appears to him that the undulating membrane is merely the edge of the fold which occurs in twisted forms. The fixation of the flagellates and the cysts was not entirely satisfactory.

7. *Family:* CERCOMONADIDÆ Kent, 1880.

This family includes flagellates which may be supposed to have originated from flagellates of the *Heteromita* type in which the trailing flagellum has become attached to the surface of the body. In addition to the attached flagellum, which is posteriorly directed, there are one or more free anteriorly directed flagella.

A. Cercomonadidæ with One Anterior Flagellum.

Genus: Cercomonas Dujardin, 1841.

The members of this genus have two flagella, which arise from the anterior end of the body. One flagellum is directed forwards as a free flagellum, while the axoneme of the other turns backwards over the surface of the body to which it is attached. It becomes a flagellum at the posterior end of the body. Though the name *Cercomonas* has been frequently used to designate intestinal flagellates of man, these have belonged to other genera, such as *Trichomonas* and *Chilomastix*. The flagellates of this genus are common in infusions, where they were first seen and named by Dujardin (1841). They also appear in old fæces as coprozoic organisms, but there is no evidence that they are ever parasitic in the human intestine. Some observers, as, for instance, Porter (1918), record them as occurring in the human stool, but it is probable

that in all these instances they had developed from cysts of free-living forms.

Cercomonas longicauda Dujardin, 1841.—The commonest form to appear in old fæces is probably *C. longicauda*, which was described by Dujardin (1841). It was seen by Klebs (1892), who referred to it as *Dimorpha longicauda*. The writer (1910) met with it in stale fæces, and maintained it in culture in hay infusion, and also on agar plates. The flagellate has a more or less pear-shaped body, which varies in length from 2 or 3 microns to as much as 18 microns. The shape of the body, which is metabolic, changes very much according to the kind of medium in which the flagellate is living. In surface films it becomes definitely amœboid. There is no cytostome, and food is ingested in an amœboid manner. A contractile vacuole has not been seen. Near the anterior region of the body is a nucleus consisting of a nuclear membrane and central karyosome. The membrane is drawn out into a cone-like prolongation, and at the apex of the cone there commences a rhizoplast formed of two closely applied axonemes, which passes to the anterior end of the body. One axoneme enters a forwardly directed flagellum, which may be two or three times the length of the body. The other passes backwards over the surface of the body to the tapering posterior extremity, where it enters a tail flagellum. At the tip of the nuclear cone, from which the axonemes arise, there is a granule which represents two minute blepharoplasts.

Reproduction is by longitudinal division (Fig 259). The first indication of this process is the formation of two new flagella from the granules at the tip of the nuclear cone, while the karyosome takes the form of a band. The granules now divide into two pairs, each of which gives origin to two flagella. The daughter granules move apart, and the nuclear membrane becomes spindle-shaped, while the band of chromatin is arranged as a plate at the equator of the spindle. The plate is made up of a number of chromosomes. By their division two plates are formed, and these pass to opposite poles of the nucleus, which now stretches right across the body of the flagellate, and has a pair of blepharoplasts at each end. The nucleus is finally divided at its centre, and this is followed by division of the flagellate.

The flagellate becomes encysted in spherical cysts, which are 6 or 7 microns in diameter. There is a central nucleus with large karyosome, while the cytoplasm is often filled with refringent granules which stain black with iron hæmatoxylin. The cysts can be dried, and will give rise to cultures again if placed in suitable medium.

Woodcock (1916) isolated *C. longicauda* from the fæces of sheep and goats. He observed the process of syngamy (Fig. 41). Two organisms

become united by their posterior ends, and gradually fuse. The nuclei unite, and at this stage the flagellate has the appearance of a uninucleate

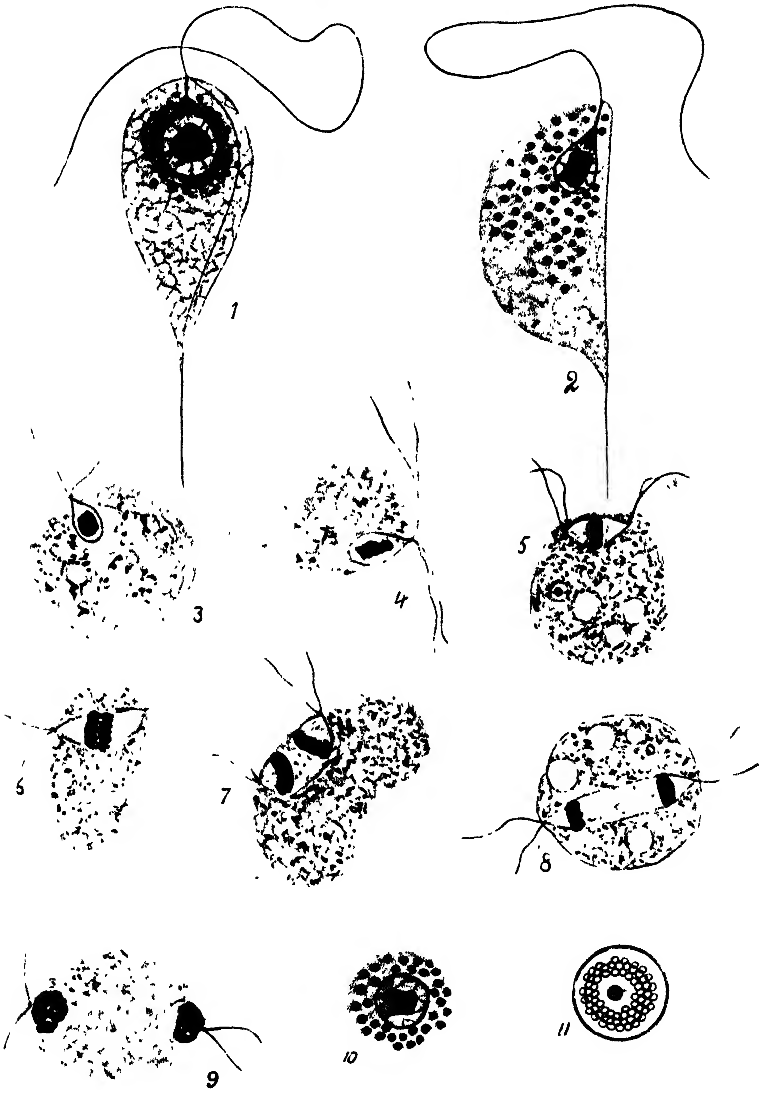


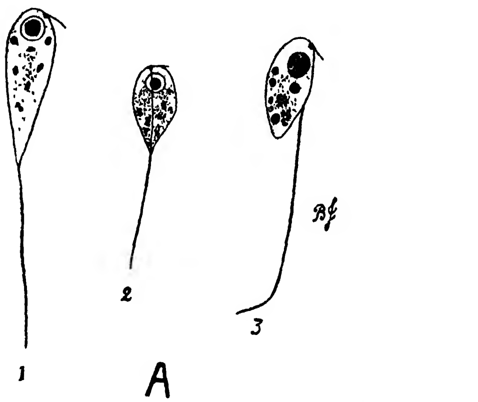
FIG. 259.—*Cercomonas longicauda* (× *ca.* 2,000). (AFTER WENYON, 1910, 1913.)

1-2. Flagellate showing attached flagellum passing over surface of body.
3-9. Various stages of division; the blepharoplast functions as a centrosome.
10. Encysted form (stained). 11. Encysted form (living).

organism with two flagella. The flagella are then lost, and encystment in a spherical cyst takes place.

Liebetanz (1910) described three species of *Cercomonas* from the stomach of cattle. These differ from one another only in size, and as no indication is given of the posterior flagellum, it seems very probable that they are merely elongate forms of the organisms which he describes as *Sphæromonas*, and which have been dealt with above.

Castellani and Chalmers (1910) described as *Heteromita zeylanica* a flagellate seen in human fæces in Ceylon. It was again recorded by Castellani (1917) from Macedonia. The statement that a flagellum existed at each end of the body shows that the flagellate does not belong to the genus *Heteromita*. These authors, however, state that they believe *Heteromita*



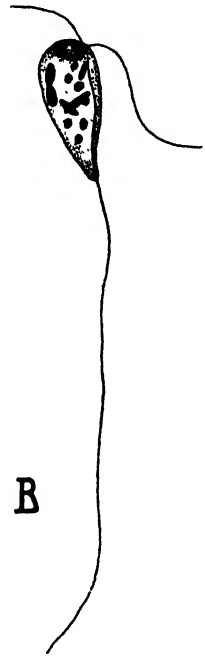


Fig. 260.

(A) Species of *Helkesimastix* Coprozoic in Goat and Sheep Dung (× 2,250). (After Woodcock, 1921.)

1. *H. major*; 2 and 3, *H. fæcicola*.

(B) *Trimitus motellæ* from the Rectum of the Marine Fish, *Motella tricirrata* (× 2,250). (After Alexeieff, 1910.)

to be a synonym of *Cercomonas*, so that it is highly probable that the flagellate was *C. longicauda* occurring coprozoically in the fæces.

Genus: **Helkesimastix** Woodcock and Lapage, 1915.

This genus was established by Woodcock and Lapage (1915) for a certain small flagellate which they encountered in cultures of goat's fæces. The original description was corrected by Woodcock (1921). The flagellate resembles *Cercomonas* in that it possesses two flagella, the axoneme of one of which is adherent to the surface of the body as far as its posterior

end (Fig. 260, A). It differs, however, in that the anterior flagellum is exceedingly short, while the posterior flagellum is about the length of the body. The axonemes appear to arise from the nuclear membrane as in *Cercomonas*. Spherical encysted forms occur, and syngamy was observed to take place by the gradual union of two individuals. Two species are described, the smaller of which, *H. fæcicola*, had an ovoid body measuring 4 to 6 microns in length.

B. Cercomonadidæ with Two Anterior Flagella.

Genus: Trimitus Alexeieff, 1910.

This genus was founded by Alexeieff (1910) for a small flagellate which had two anteriorly directed flagella and one posteriorly directed, the axoneme of which passed over the surface of the body. There is one species, *Trimitus motellæ*, which occurs in the intestine of *Motella tricirrata*, a marine fish. One of the anterior flagella is about as long as the body and the other about half this (Fig. 260, B). The posterior flagellum is a thick one, which is attached to the body as in *Cercomonas*, and has a length four or five times that of the body itself. There is a nucleus near the anterior end of the flagellate, and near it a granule in which the axonemes of the flagella originate. A similar flagellate was discovered by Duboscq and Grassé (1923, 1924) in the termite, *Calotermes flavicollis*, of France. It resembled *T. motellæ* of Alexeieff, except in the possession of an axostyle and a small rod-like parabasal which was attached to the blepharoplast (Fig. 279). They believe it possible that Alexeieff had overlooked these structures, and think that the flagellate sometimes has two and at other times three anterior flagella. In a later paper (1924*a*) they point out that the flagellate, in their opinion, is merely a young form of *Trichomonas dogieli* (see p. 675).

Chalmers and Pekkola (1919) described as *Dicercomonas sudanensis* a flagellate which they found in human fæces. The name was subsequently (1919*a*) changed by them to *Diplocercomonas sudanensis*, as the name *Dicercomonas* had been previously used (Diesing, 1865; Grassi, 1879). According to their description, the flagellate resembles *Cercomonas* with the exception that there are two anterior flagella instead of one. The writer has been able to examine the original films, and finds two flagellates are actually present. One of these is *Tricercomonas intestinalis*, described below, and the other *Embadomonas intestinalis*, and it is evidently owing to the fact that the double nature of the infection was overlooked that the presence of a new flagellate having the structure described above was accepted. There were no flagellates present which had the characters of *Diplocercomonas sudanensis*, except some examples of *Tricercomonas*, in which only two anterior flagella were visible.

C. Cercomonadidæ with Three Anterior Flagella.

Genus: **Tricercomonas** Wenyon and O'Connor, 1917.

This genus was founded by the writer and O'Connor (1917) for a flagellate having the general structure of a member of the genus *Cercomonas*, except that it possessed three anterior flagella instead of one. There is only one species.

Tricercomonas intestinalis Wenyon and O'Connor, 1917.—This is a minute pear-shaped organism which has three anterior flagella and a fourth posterior one, the axoneme of which is attached to the surface of the body. The name *T. intestinalis* was given to this flagellate by the writer and O'Connor, who discovered it in diarrhœic stools in Egypt. The writer saw the same organism later in several cases in Macedonia, while it was recorded by Kofoid, Kornhauser, and Plate (1919) and Kofoid (1920) in soldiers who had returned to New York from service abroad. Lynch (1922*a*) and Boeck (1924) have seen it in North America, Jepps (1923) in Malaya, and Da Cunha and Pacheco (1923) in Brazil.

The flagellate, which is an active metabolic organism when seen in freshly passed stool, is 4 to 8 microns in length (Fig. 261). It is pear-shaped, but the side along which the axoneme of the posterior flagellum passes is somewhat flattened. The posterior extremity is often drawn out into a tail, while the flagellum is continued for a short distance beyond the end of the tail. In stained specimens a nucleus can be seen near the anterior end of the body. It has a central karyosome, while the nuclear membrane is drawn out into a cone, from the apex of which the axonemes of the flagella originate.

Reproduction takes place by longitudinal division, but only isolated stages of this process were seen. The accounts of the flagellate given by Lynch (1922*a*) and Boeck (1924) agree in the main with that of the writer and O'Connor (1917). The organisms described by Brug (1923) and Jepps (1923) as *Enteromonas hominis* are unquestionably *T. intestinalis.* Boeck (1924), who has cultivated the organism in his L.E.S. medium from a human case, gives the measurements of the flagellate as 4 to 10 by 3 to 6 microns. The nucleus is described as spherical, and two blepharoplasts were noted near the nuclear membrane. In one of these the axonemes of the three anterior flagella originated, while the other gave origin to the axoneme of the posterior flagellum. Boeck believes that the cone-like appearance of the nucleus described by the writer and O'Connor is not a normal one. A similar cone-like arrangement occurs, however, in *Cercomonas longicauda* and *Heteromita uncinata.* In one of the cases studied by the writer and O'Connor, encysted forms of *T. intestinalis* were encountered. Cysts were also seen by Boeck. These are

oval in outline, and measure 6 to 8 microns in length by about half this in breadth (Fig. 261, 5-8). In stained films it is seen that the cysts have one, two, or four nuclei. At the four-nuclear stage the nuclei are arranged in pairs at opposite ends of the cyst. The infections which were studied in Egypt did not persist for long periods. In one case the flagellate was seen daily for nine days, when it disappeared.

It should be mentioned here that in their work on the intestinal

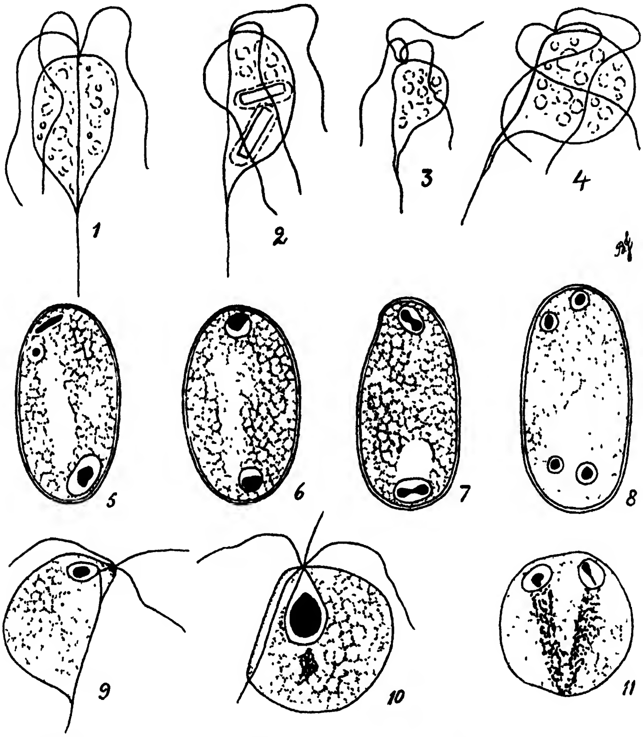


FIG. 261.—*Tricercomonas intestinalis* FROM THE HUMAN INTESTINE (× 2,600). (AFTER WENYON AND O'CONNOR, 1917.)

1-4. Flagellates as seen in living condition. 5-8. Encysted forms in stained films.
9-10. Flagellates in stained films. 11. Dividing form.

Protozoa, Dobell and O'Connor (1921) came to the conclusion that the *E. hominis* described from man by Fonseca, and which has been referred to above (p. 306), is the same as *T. intestinalis.* Jepps (1923) refers to the flagellate seen by her in Malaya as *E. hominis.* It is assumed by Dobell and O'Connor that Fonseca and other observers have overlooked the posterior flagellum, and have erroneously supposed that only three anterior flagella are present. This is, of course, quite possible, as the determination of the structure of these small flagellates is exceedingly difficult. It is, however,

possible that a flagellate of the type of *E. hominis* actually exists as a human parasite, and further observations are necessary before it is finally concluded that *Tricercomonas* is a synonym of *Enteromonas*. Lynch (1922*a*), who has studied a case of infection with *T. intestinalis* in America, has also cultivated from the intestine of the guinea-pig a flagellate having the characters of Fonseca's *E. hominis*, so the possibility of such a form occurring in man cannot be excluded.

There is another aspect of the question, which has been referred to above. In cases of infection with *Chilomastix mesnili* it is not unusual to find small rounded forms of this flagellate in which the cytostomal groove is difficult to detect (Fig. 256, 7-8). These have essentially the structure ascribed to *E. hominis* by the various observers who have recorded this flagellate. Having examined the original films, the writer is in a position to state that the flagellates described by Chalmers and Pekkola as *E. hominis* from the Sudan are actually the small round forms of *C. mesnili*. It is possible, therefore, that *Enteromonas* is a synonym of *Chilomastix*.

Duboscq and Grassé (1924), from observations on the flagellates of termites, arrive at the conclusion that both *Enteromonas* and *Tricercomonas* are merely young forms of other flagellates. In the case of *Enteromonas*, as pointed out above, there is considerable evidence in favour of this view, but in the case of *Tricercomonas* of man there is no indication whatever that it is a young form of any other flagellate. The form in the termites which Duboscq and Grassé considered as of the *Tricercomonas* type possessed both an axostyle and a parabasal (Fig. 279), in which respects it differed from *T. intestinalis* from the human intestine.

Boeck (1924), and Thomson, J. G. and Robertson (1925), report the cultivation of *T. intestinalis* in Boeck's L.E.S. medium.

8. *Family:* CRYPTOBIIDÆ Poche, 1913.

The flagellates which are included in this family are found in three situations—viz., the blood of fish, the intestinal canal of fish and the Chætognathan *Sagitta*, and the vesicula seminalis and spermatophores of molluscs and other invertebrates. Structurally, the flagellates from these three situations are so similar to one another that many observers regard them as belonging to a single genus. As will be explained below, the correct name of the genus is *Cryptobia* Leidy, 1846, and strictly all the forms should be included in this genus. The blood-inhabiting forms have so long been known under the name *Trypanoplasma*, given them by Laveran and Mesnil (1901*c*), that it seems better at present to retain them as a distinct genus.

The body of a typical member of the genus consists of an elongate flattened portion of cytoplasm in which is a nucleus and a kinetoplast

consisting of a parabasal body and two blepharoplasts. From each blepharoplast arises an axoneme which passes through the cytoplasm as a rhizoplast to the anterior end of the body. Here one enters a flagellum, which is directed forwards, while the other passes backwards along the border of an undulating membrane to become a flagellum at the posterior end of the body. The question of the possible origin of trypanosomes from these forms by the loss of the anteriorly directed flagellum has been discussed above (p. 316). It seems very improbable that trypanosomes have originated in this way. The primitive type from which they have been evolved is presumably a flagellate of the leptomonas form seen typically in insects, while the forms now being considered seem to have sprung from *Bodo* or *Cercomonas* ancestors. In fact, the members of this family are structurally very like species of *Bodo* and *Cercomonas*. From the former they differ in the absence of the cytostome, and in the backwardly directed axoneme being attached to an undulating membrane, while from the latter they differ in the possession of a kinetoplast. It is probable that the forms which occur in the blood of fish have been derived from intestinal forms which have invaded the blood-stream.

In association with a blood habitat a method of transmission through the agency of leeches has been evolved, while the purely intestinal forms are presumably handed on directly from fish to fish by the ingestion of forms which escape in the fæces. The molluscan forms, which live in the vesicula seminalis or spermatophores, are probably transmitted directly from host to host during copulation.

It will be most convenient to consider these flagellates under the following headings—Invertebrate Forms, Intestinal Forms of Fish, and Blood Forms of Fish.

A. Invertebrate Forms.

The first flagellate of this family to be seen was described by Leidy (1846) from the sexual organs of various species of snail (*Helix*) in America. He named the flagellate *Cryptobia helicis*, but in the following year (1847) renamed it *Cryptoicus helicis*, as the name *Cryptobium* had been previously employed for a beetle. Diesing (1851) referred to it as *Bodo helicis*, while Leidy (1851 and 1856) accepted Diesing's conclusion that it belonged to Ehrenberg's genus *Bodo*. It was found by Keferstein and Ehlers (1860) in *Helix pomatia* in Germany, and was studied in detail by Friedrich (1909). It was further studied by Jollos (1910), Crawley (1909), and by Delanoë (quoted by Laveran and Mesnil, 1912) in France in *H. pomatia*, *H. hortensis*, and *H. nemoralis*, and by Bělař (1916). It is evidently a common parasite of the various species of *Helix* in America and Europe. The correct name of this organism is clearly *Cryptobia helicis* Leidy, 1846,

though most writers have placed it in Laveran and Mesnil's genus, *Trypanoplasma*, which was established by them in 1901. According to the rules of nomenclature, Leidy had no necessity to change the name to *Cryptoicus*.

Cryptobia helicis Leidy, 1846.—According to Bělař (1916), *C. helicis* is typically an elongate organism varying in length from 6 to 20 microns (Fig. 262). The breadth varies considerably, there being comparatively narrow forms not more than 3 microns in breadth and others which are much broader. Typically, however, the organism has an elongate form. The body is distinctly flattened. There is a nucleus consisting of a nuclear membrane enclosing a space in which there is a central karyosome and a number of scattered chromatin granules. Nearer the anterior end is the kinetoplast, consisting of an elongate parabasal body and two blepharoplasts, which are often so close together as to appear as one. From each there arises an axoneme. One of these passes through the cytoplasm to the anterior end of the body, where it becomes a flagellum, which is about as long as the body itself. The other passes to the surface of the body in a lateral or backward direction, and then runs over the surface of the body, to which it is adherent, as far as the posterior extremity. It is then continued in a short flagellum. There does not appear to be an undulating membrane at the line of attachment of the axoneme.

The flagellate multiplies by binary fission. The process has been described in detail by Bělař. The single pair of blepharoplasts divides to form two pairs, the original axonemes remaining with one pair, while new axonemes commence to grow out from the other pair. As the two pairs of blepharoplasts separate, the parabasal body splits from before backwards. At the same time changes take place in the nucleus. The karyosome becomes elongated and dumb-bell-shaped, and its two halves become more and more separated, though still connected by a fibre, as the nucleus itself increases in length. The chromatin granules of the nucleus at a certain stage appear to be collected at the equator of the nucleus as an irregular plate. This plate is divided into two parts, which travel to opposite poles of the nucleus. Finally, the nucleus is divided into two. The nuclear division is thus a modified form of mitosis. By the time nuclear division is complete, the flagellate has two complete sets of organs. The body now divides, and two organisms result. Bělař described what he supposed to be conjugation, in which two flagellates unite, their nuclei and kinetoplasts fusing, but in a later paper (1924) he admits that this was an erroneous interpretation of the appearances seen by him.

1-4. Various types of flagellate. 5. Commencing division of nucleus.
6-7. Divided blepharoplast and commencing division of parabasal.
8. Blepharoplast and parabasal completely divided; nucleus dividing.
9-10. Final stage of division.

FIG. 262.—*Cryptobia helicis* FROM THE RECEPTACULUM SEMINIS OF THE SNAIL, *Helix pomatia* (× 3,800). (AFTER BĚLAŘ, 1915.)

[*For description see opposite page.*

So far, no observer has noted an encysted stage of this flagellate, though it appears evident that the flagellate is handed on from snail to snail during copulation.

Poche (1903) described a very similar flagellate from the stomach of certain Cœlenterates (*Siphonophora*). The organism was studied by Keysselitz (1904), who gave it the name *Trypanophis grobbeni*. The flagellate measures 65 by 4 microns, has a centrally placed nucleus, and a small kinetoplast which is near the anterior end of the body. There is a short anterior flagellum and another posterior one, which is continuous with an axoneme attached to the body by a narrow undulating membrane. The flagellate differs from the members of the genus *Cryptobia*, considered above, in the shortness of the anterior flagellum and the small size of the kinetoplast, but it is doubtful if this is sufficient justification for its inclusion in a distinct genus.

A flagellate, which probably belongs to the genus *Cryptobia*, has been described by Hesse (1910) from the vagina of leeches (*Hirudo medicinalis* and *Aulastomum gulo*) as *Trypanoplasma vaginalis*. *Cryptobia carinariæ* was recorded by Collin (1914) from the seminal receptacle of the mollusc *Carinaria mediterranea*. Another form, which undoubtedly belongs to the same genus, was recorded by Fantham and Porter (1910) as *T. dendrocœli*, from the intestine of the fresh-water planarian, *Dendrocœlum lacteum*. The organism was studied by Gelei (1913). Its structure and method of reproduction was very similar to that of *C. helicis*. Intracellular forms also occurred, as previously noted by Fantham and Porter, but Gelei did not observe them in the cells of the ovary, as these authors maintained. He considered the intracellular forms as being merely an indication of the phagocytic powers of the host cells. Hamburger (1912) described as a *Trypanoplasma* a flagellate of the mole cricket, *Gryllotalpa vulgaris*. Structurally, it resembled a *Cercomonas* in that there was no separate kinetoplast, and it is possible that it belongs to this genus. Under the name *Trypanoplasma isidoræ* Fantham (1923) describes a flagellate of the receptaculum seminis of the pond snail *Isidora tropica* in South Africa.

B. Intestinal Forms of Fish.

The first flagellate of this type to be seen in the intestine of marine fish was one discovered by Dahl in 1887 in *Cyclopterus lumpus*. It was referred to by Möbius (1888) as *Diplomastix dahlii*. Keysselitz (1906) named it *Trypanoplasma ventricoli*, as Léger, L. (1905), had placed in this genus as *T. intestinalis* a similar flagellate from the marine fish, *Box boops* (Fig. 263).

Elmhirst and Martin (1910) gave the name *T. congeri* to a form from the stomach of the conger eel, *Conger niger*. Its method of multiplication

by binary fission was described by Martin (1910). Alexeieff (1910) encountered *T. intestinalis*, not only in *B. boops*, but also in *Motella tricirrata*. Apstein (1910) studied the form in *Cyclopterus*, and described it as *Heteromita dahlii*.

According to these authors, the various flagellates have essentially the same structure as *Cryptobia helicis*. Alexeieff gives the measurements of *T. intestinalis* as 14 to 18 microns by 3 to 5 microns. The anterior flagellum measures about 14 microns, while the posteriorly directed flagellum, the axoneme of which is attached to the border of an undulating membrane, measures about 28 microns.

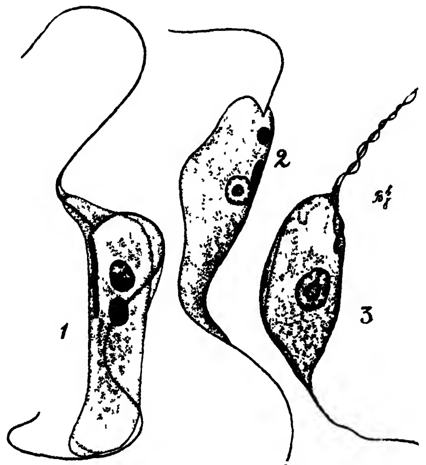


FIG. 263.—INTESTINAL TRYPANOPLASMS OF FISH. (AFTER MARTIN, 1913.)

1. *Cryptobia congeri* from the stomach of the conger eel (*Conger niger*) (× *ca* 1,800).
2. *Cryptobia dahlii* from the stomach of *Cyclopterus lumpus* (× *ca.* 1,200).
3. *Cryptobia* (*Trypanoplasmoides*) *intestinalis* from stomach of *Box boops* (× *ca.* 1,800).

Martin (1913) published an account of the flagellates from these three fish. He was convinced that the form named *T. intestinalis* has three anterior flagella, which are often twisted together so closely that observers have erroneously concluded that only one flagellum is present. Accordingly, Martin places it in a new genus as *Trypanoplasmoides intestinalis*. Structurally, according to him, it resembles *C. helicis*, except that it possesses three anterior flagella instead of a single one. As regards the flagellate of *Cyclopterus*, first seen by Dahl, Martin states that a small cytostome is present at the base of the flagella, the axoneme of one of which passes backwards over the surface of the body, to which it is attached, without there being any evidence of an undulating membrane. For these reasons he follows Apstein (1910) in retaining it in the genus *Heteromita* as *H. dahlii*. It certainly does not belong to this genus, the members of which have two free flagella and axonemes arising from blepharoplasts on the nuclear membrane (Fig. 142). It is better to retain it in the genus *Cryptobia* at present, as also the form in the conger eel, for which Martin retains the name *Trypanoplasma congeri*. Woodcock and Lodge (1921) described as *C. trematomi* a flagellate which was found in the stomach and intestine of a fish (*Trematomus bernacchii*).

Walker (1910) stated that he had obtained a culture of a trypanoplasm from the intestine of the frog, *Rana palustris*, by inoculating agar plates. It

seems more probable that he was dealing with some other flagellate, possibly a *Cercomonas*. Hovasse (1924) has given the name *Trypanoplasma sagittæ* to a form he has found in the intestine of a little marine worm belonging to the genus *Sagitta*.

C. Blood Forms of Fish.

Laveran and Mesnil (1901*c*) created the genus *Trypanoplasma* for a flagellate they discovered in the blood of the fresh-water rudd, *Leuciscus erythrophthalmus*, which they named *T. borreli* (Fig. 151). Since that date, a number of similar forms have been discovered in the blood of fresh-water fish. Curiously enough, no one has yet described an intestinal form from fresh-water fish, though such forms have been seen in marine fish, in which, however, the blood forms do not occur. Structurally, the blood flagellates are similar to those of snails and the intestine of marine fish, so that strictly they should be included in the genus *Cryptobia*. The blood forms have been so long known by the name *Trypanoplasma* that it seems better to retain this name for them provisionally. They are carried from host to host by leeches, while the other forms have a different method of transmission. In the case of the intestinal flagellates of fish, and possibly those of snails, it might be expected that encysted forms would occur. Such, however, have not yet been described. Future investigation may, however, reveal encysted forms, in which case the retention of the name *Trypanoplasma* for the blood forms would be justified. The possibility of a difference in life-cycle was suggested by Woodcock and Lodge (1921)

The various trypanoplasms of fish resemble one another very closely, so much so that Keysselitz (1906), who studied these flagellates in many species of fresh-water fish, came to the conclusion that they all belonged to the species *T. borreli*. Other observers, however, have given specific names to the forms occurring in different fish.

The following species have been described:

T. borreli Laveran and Mesnil, 1901: *Leuciscus erythrophthalmus* (rudd).
T. cyprini Plehn, 1903: *Carassius vulgaris* (carp), *C. auratius* (goldfish).
T. varium Léger, 1904: *Cobitus barbatula* (loach).
T. guernei Brumpt, 1905: *Cottus gobio* (bull-head).
T. barbi Brumpt, 1906: *Barbus fluviatilis* (barbel).
T. abramidis Brumpt, 1906: *Abramis brama* (bream).
T. truttæ Brumpt, 1906: *Salmo fario* (trout)=*T. valentini* Gauthier, 1920: *Salmo fario*.
T. sp. Rodhain, 1907: *Labeo macrostoma*
T. gurneyorum Minchin, 1909: *Esox lucius* (pike).
T. clariæ Mathis and Leger, 1911: *Clarias macrocephalus*.
T. sp. Mathis and Leger, 1911: *Monopterus javanensis*.
T. keysselitzi Minchin, 1909: *Tinca tinca* (tench).
T. sp. Tanabe, 1924: *Misgurnus anguillicaudatus*.
T. ninæ kohl-yakimov Yakimoff, 1925: *Silurus glanis*.

Keysselitz (1906) published an account of the trypanoplasmas of fish which he had seen in *Perca fluviatilis*, *Acerina cernua*, *Lota vulgaris*, *Barbus fluviatilis*, *Cyprinus carpio*, *Carassius vulgaris*, *Tinca tinca*, *Abramis brama*, *Leuciscus idus* (*Idus melanotus*), *L. cephalus* (*Squalius cephalus*), *L. erythrophthalmus* (*Scardinius erythrophthalmus*), *L. rutilus*, *Esox lucius*, and *Cobitis barbatula.* He regarded them as all belonging to the one species, *T. borreli*, to which Léger, L. (1904*f*), had ascribed a form seen by him in the minnow, *Phoxinus lævis.*

The various species described differ from one another merely in their dimensions, in the position of the nucleus, and other details. The parasites are usually scanty in the blood of fish, so that in most cases the observations have been made on only a few individuals. It is impossible to be sure, therefore, that the forms do not belong to one species, as Keysselitz maintains.

Trypanoplasma borreli Laveran and Mesnil, 1901.—This flagellate, as described by Laveran and Mesnil, is a flattened elongate organism with a rounded anterior end, and usually a somewhat pointed posterior end (Fig. 151). It is distinctively curved, so that one side of the flattened body is convex and the other concave. The nucleus lies just behind the anterior third of the body near the convex border. In properly fixed specimens it is seen to consist of a nuclear membrane and central karyosome. Opposite the nucleus, and near the concave border, is the kinetoplast, consisting of an elongate parabasal, just anterior to which are two closely applied blepharoplasts. From one of these arises an axoneme, which passes forwards round the anterior end of the body, and thence backwards on the edge of the undulating membrane along the convex border to the posterior end, where it becomes a flagellum. From the other blepharoplast there arises an axoneme, which passes into the anteriorly directed flagellum. The body of the flagellate is about 20 microns in length by 3 to 4 microns in breadth. The two flagella are about 15 microns in length. Slightly larger or smaller forms occur, while in preparations many curious distorted flagellates result from the metabolic nature of the body. Reproduction in the blood of the fish is by longitudinal division, and follows very closely the process as described above for *Cryptobia helicis.*

The trypanoplasm of the rudd is not only inoculable to uninfected rudd, but also to minnows, as proved by Laveran (1904*b*). Similarly, the naturally occurring trypanoplasm of minnows is inoculable to rudd. Plehn (1903) succeeded in inoculating the trypanoplasm of carp to other carp.

Ponselle (1913) has succeeded in cultivating the trypanoplasm (*T. varium*) of the loach. The medium employed consisted of a 2 per cent. agar in tap water without the addition of salt, to which one volume of

defibrinated rabbit's blood was added as in N.N.N. medium. The cultural forms resembled the blood flagellates, except that the undulating membrane was less developed. In some the posterior flagellum was detached as a trailing flagellum, giving the organism a likeness to species of *Bodo*.

According to Plehn (1903) and Keysselitz (1906), the trypanoplasms are liable to produce various morbid symptoms in the fish, which appear paler than normal fish do, and may show œdematous swellings of the body. In some cases there is marked loss of vitality, terminating in death.

Transmission. — In nature the trypanosplasms are transmitted from one fish to another by leeches. Léger, L. (1904*e*), noticed that numerous trypanoplasms occurred in the intestine of leeches (*Hemiclepsis marginata*) which had fed on infected fish (Fig. 240). He also saw small forms in a species of *Piscicola* which had fed on infected minnows. Brumpt (1906*b*) found that *T. guernei* of the bull-head and *T. barbi* of the barbel multiplied in the intestine of *Piscicola*, while *T. abramidis* of the bream developed in *H. marginata*. The question of transmission was studied more completely by Keyssilitz (1906) in *P. geometra* (Fig. 245). According to him, there is at first a conjugation in the crop of the leech of forms which he regarded as gametes. The zygote thus formed has no flagella, but is an ovoid body containing a nucleus and kinetoplast, each of which is supposed to be the result of fusion of the corresponding structures of the gametes. No confirmation of this process has yet appeared. In the crop of the leech there is active multiplication of the flagellates by fission till a large number is present. These vary very much in size and shape, but there is a tendency towards the production of small slender forms, which eventually make their way into the proboscis sheath. It is presumably these slender proboscis sheath forms which enter the wound inflicted by the leech in the act of feeding. Multiplication was also noted to take place for a short period in *Hirudo medicinalis*.

Robertson (1911) studied the development of the trypanoplasm of gold fish in England in *Hemiclepsis marginata* and *Piscicola* sp. (Fig. 264). About four to five hours after a young leech had fed, dividing trypanoplasms could be seen in the crop. These appear to be somewhat broader than the blood forms which were ingested. Multiplication proceeds till, on the second day, slender comma-shaped forms make their appearance. All intermediate types between these and the broad forms are still present. After some days the slender forms move forwards to the proboscis and

1. Form in blood of goldfish. 2. From crop of leech forty-four hours after feeding. 3. From crop three and a half days after feeding. 4. From crop six days after feeding. 5. From crop six days after feeding. 6. From crop seven days after feeding. 7. From crop ten days after feeding. 8. From crop twenty-five days after feeding.

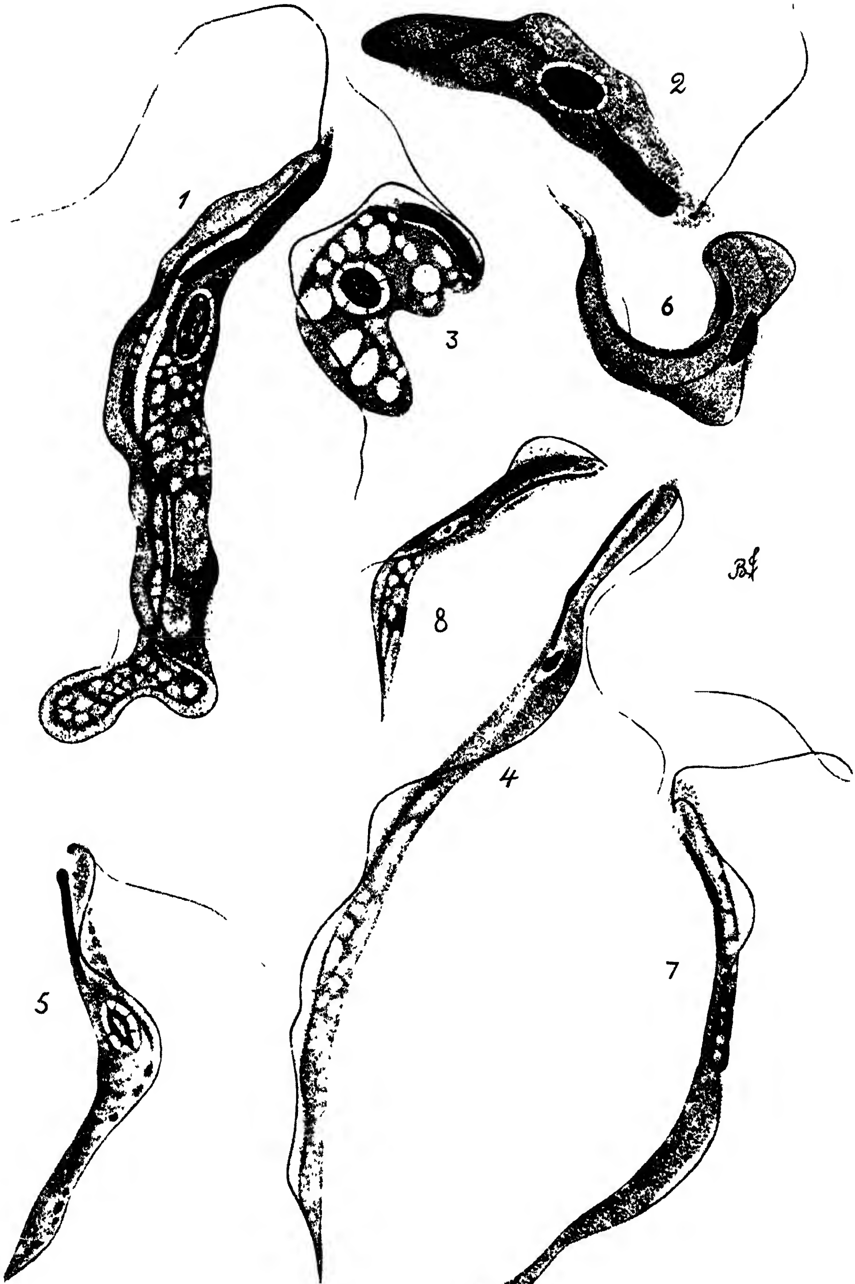


FIG. 264.—*Trypanoplasma cyprini* FROM THE BLOOD OF THE GOLDFISH AND THE INTESTINE OF THE LEECH, *Hemiclepsis marginata* (× 4,000). (AFTER ROBERTSON, 1911.)

[*For description see opposite page.*

enter the sheath, in which situation they may be seen in incredible numbers, either free or attached to the wall of the sheath by their flagella. By the end of the tenth day, before which the leech is not ready to feed again, there are numerous flagellates in the proboscis sheath and anterior part of the crop, but none farther back. Exceptionally, the fresh feed will clear the leech of flagellates entirely, but, as a rule, the proboscis sheath, which is emptied at the feed, becomes filled with the slender forms which have resulted from multiplication of those left in the sheath or by a further migration forwards from the crop. A fish, upon which infected leeches had fed, first showed trypanoplasms in its blood on the seventh day. According to Brumpt (1906*b*), the trypanoplasms with which he worked multiply in the gut, but do not invade the proboscis sheath. Tanabe (1924) has given an account of the development of the trypanoplasm of the Japanese loach (*Misgurnus anguillicaudatus*) in the leech, *Hirudo nipponica*. In the intestine active multiplication occurred during the first three days, so that large numbers of small forms were produced. After this there was a gradual disappearance of the flagellates, though in some cases they persisted for eight or nine days. No mention is made of any attempts to transmit the infection by means of these leeches.

9. *Family:* TRICHOMONADIDÆ.

The flagellates belonging to this family are characterized by the possession of a variable number of flagella, a definite cytostome, and a rod-like structure, the axostyle, which arises from the blepharoplasts and passes through the body to its posterior end, through which it usually protrudes. In some forms, one of the flagella is directed backwards, and its axoneme may be attached to the border of an undulating membrane. In such cases there is usually a stiff basal fibre, which lies along the line of attachment of the undulating membrane to the body (Fig. 265). The family includes the following genera:

Genus: **Trichomonas** Donné, 1837.
,, **Ditrichomonas** Cutler, 1919.
,, **Gigantomonas** Dogiel, 1916.
,, **Eutrichomastix** Kofoid and Swezy, 1915.
,, **Janickiella** Duboscq and Grassi, 1923.
,, **Trichomitus** Kofoid and Swezy, 1919.
,, **Devescovina** Foa, 1905.
,, **Foaina** Janicki, 1915.
,, **Retortamonas** Grassi, 1879.
,, **Protrichomonas** Alexeieff, 1911.
,, **Polymastix** Bütschli, 1883.
,, **Hexamastix** Alexeieff, 1912.
,, **Cochlosoma** Kotlan, 1923.

It must, however, be mentioned that flagellates of the genus *Trichomonas* are very liable to exhibit changes of structure, so that they appear to resemble members of another genus. By detachment of the membrane flagellum a flagellate of genus *Eutrichomastix* is simulated. The genus *Hexamastix* could be accounted for by supposing that the membrane flagellum of a *Trichomonas* with five anterior flagella (*Pentatrichomonas*) had become detached from the membrane, so as to give rise to forms with six anterior flagella and no undulating membrane. The axostyle and the supporting fibre of the undulating membrane may be difficult to detect, so that forms with a number of flagella and no other structures result. It has been shown above (p. 310) that the genus *Protetramitus* was founded on what were merely altered forms of *Trichomonas* or *Eutrichomastix*. It is not improbable that the genus *Trichomitus* was similarly established for flagellates which actually belong to the genus *Trichomonas*, and in which the axostyle was for some reason invisible, as not infrequently occurs with undoubted *Trichomonas*. When such forms are encountered in films, careful search will usually disclose a series connecting them with the typical unaltered *Trichomonas*. In practically every film of material containing *Trichomonas* there occur rounded cytoplasmic bodies possessing a nucleus and blepharoplasts, but with no other structures visible, similar rounded forms in which the flagella can be seen, others with the axostyle evident, and, finally, the typical *Trichomonas* with the undulating membrane and its attached flagellum. Under these circumstances the determination of genera, which may be regarded

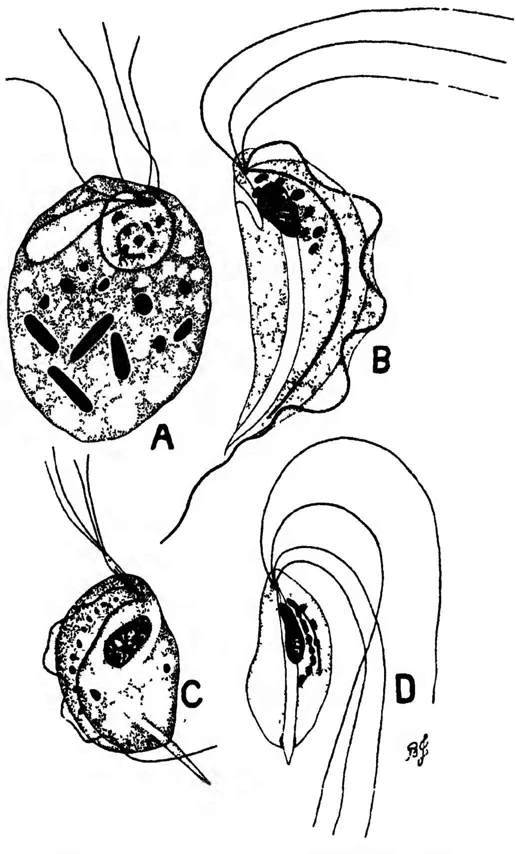


FIG. 265.—FLAGELLATES FROM THE CÆCUM OF THE FOWL (× 4,000). (AFTER MARTIN AND ROBERTSON, 1911.)

A. *Chilomastix gallinarum.*
B. *Trichomonas eberthi.*
C. *Trichomonas gallinarum.*
D. *Eutrichomastix gallinarum*

as modified *Trichomonas*, is an exceedingly difficult one, and no definite decision can be reached unless it is clearly established that the characters are constant.

Genus: Trichomonas Donné, 1837.

The flagellates of this genus have more or less pear-shaped bodies, three to five anterior flagella, and a recurrent axoneme, which is attached to the border of an undulating membrane. The axoneme may or may not be continued beyond the membrane as a posterior flagellum. There is a definite cytostome near the base of the flagella. An axostyle is present, and also a fibre which runs along the line of attachment of the undulating membrane. A nucleus is situated at the anterior end of the body, and anterior to it is a group of blepharoplasts, from which the flagella and other structures arise. In some forms a parabasal has been described in the cytoplasm between the nucleus and basal fibre of the undulating membrane. Reproduction is by binary fission, and encystment also occurs.

TRICHOMONAS IN MAN.

There is a large number of species of this genus, which differ from one another in size, shape of the body, and the number of flagella. It appears that at least three occur in man: *T. hominis* of the intestine, *T. elongata* of the mouth, and *T. vaginalis* of the vagina. It cannot, however, be considered as definitely established that these are distinct species.

Trichomonas hominis (Davaine, 1860).—This common intestinal flagellate of man was first recorded by Davaine in 1854 as *Cercomonas.* In 1860 he gave a figure and more detailed description of the organism under the name *Cercomonas hominis.* It was later placed in Donné's genus, *Trichomonas*, by Leuckart (1879) and others, and is now generally known as *T. hominis.* As noted above (p. 621), Moquin-Tandon in the year 1860 had already proposed the name *C. obliqua* for a flagellate usually regarded as a *Trichomonas,* so that, according to rule, the name should be *T. obliqua* if there is no doubt as to the identity of the organism named by him. Grassi (1879*a*, 1881*a*) referred to it as *Monocercomonas hominis.* On account of these difficulties of nomenclature, Stiles (1902) proposed the new name *Trichomonas confusa.*

T. hominis is probably the commonest intestinal flagellate of man (Fig. 266). As a rule, it is only seen in diarrhœic stools, but that it is still present when the stools are formed can be demonstrated by administering saline purges, or by the inoculation of fæces into certain media, as, for instance, Hogue's egg medium, and incubating at 24° C. for a few days, as advocated by Hegner and Becker (1922) and Reichenow (1923). Its occurrence in diarrhœa, however, is due most probably to the fact that it is only when the stools are liquid that the flagellate in its active condition

is swept out of the intestine. Its presence is not necessarily an indication that it is the cause of any intestinal derangement which may exist. It is a pear-shaped organism measuring 5 to 15 microns in length. Occasionally longer forms are seen. The shape of the body, which is normally pear-shaped, changes considerably from time to time, and under certain conditions pseudopodia are formed. The anterior end of the body is somewhat bluntly pointed, while the posterior end is more tapering and terminates in the protruding axostyle. The flagella, which are as long as

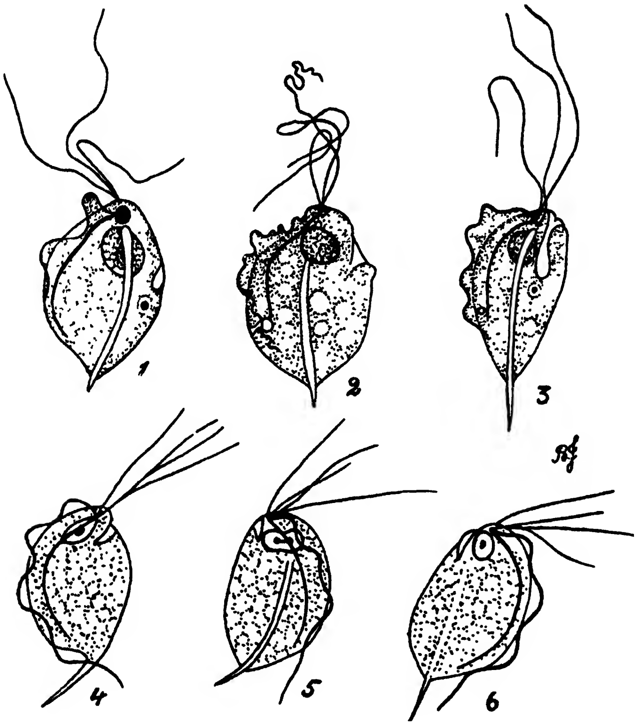


FIG. 266.—*Trichomonas hominis* FROM THE HUMAN INTESTINE (×*ca.* 2,000.) (1-3, AFTER FAUST, 1921; 4-6, AFTER WENYON AND O'CONNOR, 1917.)

1-3. Forms with three anterior flagella (*Tritrichomonas*).
4-6. Forms with four anterior flagella (*Trichomonas*).

or longer than the body, arise from the anterior extremity. These are usually four in number. They move about from one side of the body to the other, performing sweeping movements very much like the action of a whip which is lashed to and fro. Very frequently the proximal portions of the flagella appear adherent to one another or twisted to form a common stem. On the ventral side of the base of the flagella is a slit-like cytostome. There is a well-developed undulating membrane, which passes in a slightly

spiral manner along the dorsal surface to the posterior end of the body. The posteriorly directed axoneme is attached to the border of the membrane, and may be continued beyond it posteriorly for a short distance as a flagellum. The extension beyond the membrane of a flagellum does not appear to be a constant feature. In some cases it is exceedingly difficult to detect such a flagellum, especially if it is a short one, as is usually the case. Faust (1921) regards the axoneme as terminating at the end of the membrane (Fig. 266, 1-3). The membrane is in constant motion, while the anterior flagella are sweeping over the body, first on one side and then on the other. The flagellate progresses in a jerky manner, revolving continuously on its longitudinal axis owing to the action of its membrane. When observed for any length of time, many of the flagellates in a specimen become degenerate. Various changes may take place, all of which may lead to misconceptions as to the character of the organism. The axoneme may become detached from the membrane and lash about, so that if the other finer flagella are overlooked, as is easily done, the organism may be regarded as having only one long thick flagellum. Such forms have probably been mistaken for *Cercomonas*. The flagellate may lose its membrane flagellum entirely, while the cytoplasm at the anterior part of the body throws out quite suddenly a long finger-like pseudopodium, which travels backwards and at the same time becomes shorter. When it reaches the posterior end of the body it vanishes, while another one is formed again at the anterior end. These finger-like pseudopodia pass down the body regularly, and may be regarded as a series of high narrow waves, resulting from uncontrolled action of the membrane which has been deprived of its axoneme. The movements are so peculiar that Castellani (1905) was misled into describing this form as a new amœba (*Entamœba undulans*). The posterior end of the body may become swollen, so that the flagellate appears to have a spherical mass attached to it by a narrow neck. This mass of cytoplasm may be broken off. In normal individuals it is only the tip of the axostyle that protrudes from the body, but in many degenerating forms, possibly as a result of retraction of the cytoplasm or actual separation of portions of it, a greater length of axostyle is exposed. If the flagellates do not degenerate, they gradually become rounded and perfectly quiescent. In this condition the membrane with its attached axoneme passes round the circumference of the now spherical body, while the axostyle and basal fibre are curved within it. The anterior flagella may entirely disappear. It is these rounded flagellates which are suspected of proceeding to encystment, but encysted stages of *T. hominis* have not been seen. In *T. caviæ* of the guinea-pig, however, it is the spherical forms of this type which become encysted, and this appears to be true also of *T. muris* of mice. Prowazek (1904*a*) and

Bohne and Prowazek (1908) described a *Blastocystis* as being the cyst of *T. hominis*, and supposed that this encystment was associated with a process of autogamy. Several writers, including Bensen (1909), claim to have confirmed this observation. There is no doubt, however, that *Blastocystis*, a vegetable organism which can be cultivated, has no connection whatever with *T. hominis* (Fig. 118). The cysts described by Lynch (1916) are so similar to the cysts of *Chilomastix mesnili* that they cannot be regarded as cysts of *T. hominis* till further evidence has been produced. Lynch (1915*c*) described, both from fæces and cultures, spherical cysts with granular contents, but from the description it is impossible to conclude that he was dealing with encysted *Trichomonas*. They did not show any of the characters of the encysted forms as seen in animals. In a later paper this author (1922) admits that cysts of *T. hominis* have not yet been discovered.

T. hominis reproduces by longitudinal division. The process has not been studied in detail in this species, which is usually of small size. As regards the division of other species, there are many conflicting statements as to what actually occurs. In stained films, a few further details of the structure of *T. hominis* can be made out. The human flagellate is a very small form which easily shrinks on fixation, so that it is exceedingly difficult to make satisfactory preparations. In addition to the various details which can be detected in the living organisms, it can be seen that there is a spherical or slightly ovoid nucleus near the anterior end of the body. It consists of a nuclear membrane surrounding a clear space, at the centre of which is a karyosome. Anterior to the nucleus is a closely packed group of blepharoplasts. From these arise the axonemes of the anterior flagella, and also the one which borders the undulating membrane, as well as a stiff fibre, the basal fibre, which passes through the body just below the attachment of the undulating membrane. Posteriorly, the basal fibre tapers to a point. Parallel and close to it can sometimes be distinguished a row of deeply staining granules.

The axostyle is another structure which commences at the blepharoplasts. It has the form of a broad bar which takes a straight course through the body to the posterior extremity, through which it protrudes as a sharp-pointed caudal process. The axostyle, unlike the basal fibre, does not stain black with iron hæmatoxylin. In the living organism the axostyle is perfectly passive, and only moves with the contractions of the cytoplasm around it.

T. hominis feeds by ingesting bacteria through its cytostome, and these can be seen in various food vacuoles. It is possible that it also absorbs nourishment in solution through the surface of its body. Sometimes red blood-corpuscles are present in vacuoles. The writer has seen them within *T. hominis* in cases of bacillary dysentery when many red cells are present

in the stool, and also in cultures in media containing rabbit blood. The presence of included red cells has been advanced as an argument in favour of the pathogenicity of this flagellate, but there is no reason to suppose that they have been taken up by the flagellates anywhere than in the lumen of the gut. In the case of *Entamœba histolytica*, it is probable that the red cells are ingested by the amœbæ while they are still in the tissues.

Cultivation.—Several observers have cultivated *T. hominis*. Escomel (1913) stated that he had obtained a culture in a vegetable medium, but there was no evidence that he had done anything more than keep the flagellates alive, as sometimes happens, for many days in the liquid fæces themselves. Lynch (1915*a*, 1915*c*) was able to keep *T. hominis*, as well as the other human species, alive for some days in acid bouillon. In the case of *T. hominis* a few subcultures were made, and it seemed evident that multiplication had taken place. Boyd (1918, 1919) was more successful by using a saline suspension of fresh fæces in which *T. hominis* was taken through seven subcultures during sixty-five days. Ohira and Noguchi (1917) cultivated *T. hominis* (? *T. elongata*) in a mixture of ascitic fluid and Ringer's solution. Active multiplication took place in the deeper parts of this medium and by subculture every forty-eight hours when the tubes were kept at 23° to 27° C., or every twenty-four hours when, at 37° C., the flagellates were kept alive indefinitely. Barret, who has successfully cultivated *Balantidium coli* and *Blastocystis* in a 10 per cent. solution of inactivated human blood-serum in 0·5 per cent. sodium chloride solution, informs the writer that he has cultivated *T. hominis* in this medium and taken it through thirty subcultures. The writer has successfully cultivated the flagellate and maintained it by subculture in Hogue's egg medium at a temperature of 24° C. Bacterial growth takes place rapidly, and to keep the flagellates alive it is necessary to subculture every week. At higher temperatures subculture must be made more frequently. The culture method has been used for diagnosis purposes by Hegner and Becker (1922) and Reichenow (1923). Boeck and Drbohlav (1925), and Thomson, J. G. and Robertson (1925), have cultivated *T. hominis* in Boeck's L.E.S. medium.

Method of Infection.—The question of the method of infection with *T. hominis* presents some difficulties. Definite encysted forms have not been discovered in man. If infection occurs from man to man, and if it is true that cysts do not occur, then it must be assumed that infection takes place by ingestion of the active flagellates themselves, which are known to survive for a considerable time outside the body. It was shown by the writer and O'Connor (1917) in Egypt that flies fed on fæces containing *T. hominis* deposited in their dejecta live flagellates five minutes later. In this manner, food or drink could readily become contaminated with the living organisms.

Infection of Animals.—Several observers claim to have infected animals with *T. hominis*. Thus, Escomel (1913) stated that he had succeeded in infecting the rabbit, guinea-pig, dog, and cat. Lynch (1915*a*) thought he had infected rabbits by injecting them *per rectum* with cultures, but in a later paper (1922) he doubts his original claim, owing to the difficulty he has had in positively excluding a previous infection in animals. Boyd (1919) stated that he had infected a rat by feeding it with cultures of *Trichomonas*. Owing to the doubt which attaches to these experiments, Hogue (1922) attempted to infect cats, kittens, and rabbits which had, by repeated examinations, been proved to be free from *Trichomonas* infections. The animals were fed and injected *per rectum* with cultures of the flagellates, but in no case did an infection result. Kessel (1924*a*) states that he has infected monkeys.

Pathogenicity.—The fact that *T. hominis* occurs most commonly in diarrhœic stools has led observers to regard it as a pathogenic organism. When there is no diarrhœa, the flagellate is still present, though it is only rarely found in formed stools. As the cysts are not known, its presence can be recognized only when diarrhœa occurs or after the administration of a purge. It frequently happens that individuals suffer from a chronic looseness of the bowel, and that no explanation of this condition can be discovered. In a certain number of such cases *T. hominis* is present in the stools, and many clinicians assume that they are the exciting cause of the disorder. As there are many cases of this type in which flagellates cannot be discovered, it is quite illogical to assume that the few in which they are present owe their condition to these organisms.

The writer (1920) examined by section post-mortem material from five cases of *T. hominis* infection. In one of these the large intestine showed the flagellates in the lumen of the glands, actually breaking through the glandular cells, and distributed in the interglandular connective tissue (Fig. 267). Whether this invasion of the tissues of the intestinal wall is an indication of pathogenicity or not cannot be stated. It is possible that the *Trichomonas* invaded the tissues shortly before or even after death. It may be mentioned, however, that guinea-pigs, which are commonly infected with *T. caviæ*, often show ulceration of the large intestine and cæcum, with definite invasion of the tissues by the flagellate in perfectly fresh material which has been taken from animals which have been killed.

That invasion can occur at other times is borne out by an observation made by Pentimalli (1923). Examining films made from blood taken from a patient's vein under aseptic conditions, he found *Trichomonas* present. A further examination made ten hours later showed the same organism in smaller numbers. Further examinations were negative. No information as to the presence or absence of an intestinal infection was obtained.

Kessel (1925) has seen the flagellate in pus from an amœbic abscess of the liver.

T. hominis infections may be very persistent. In Egypt, the writer and O'Connor studied a case in which the flagellates were present during an observation of sixty-two days. Cases are on record, however, in which they are known to have been present for many years.

FIG. 267.—SECTION OF HUMAN LARGE INTESTINE, SHOWING INVASION OF THE WALL BY *Trichomonas hominis* (× *ca.* 1,000). (AFTER WENYON, 1920.)

The flagellates pass through gaps in the lining cells of the intestinal glands into the surrounding connective tissue.

VARIETIES OF TRICHOMONAS HOMINIS.—As already pointed out, *T. hominis* usually possesses four flagella. In any infection it will be seen that the great majority of the flagellates have a definite number of flagella, so that it seems clear that for any particular form the number is constant. It is known, however, that in certain cases the majority of flagellates have five flagella, and in other cases three (Figs. 26, 266, 289). Some observers have given special generic names, according to the number of flagella. Thus, the four-flagellate type has been called *Tetratrichomonas* by Parisi (1910), the five-flagellate type *Pentatrichomonas* by Mesnil (1914) and

Chatterjee (1915), and the three-flagellate type *Tritrichomonas* by Kofoid (1920). It seems doubtful if these forms should be placed in different genera. The type species of *Trichomonas* is *T. vaginalis* Donné (1837), and this form has always been seen to possess four flagella, though it cannot be affirmed that the three or five flagellate types never occur in the vagina. *Trichomonas hominis*, therefore, would be the correct name for the intestinal form with four flagella, there being no need to employ the generic title *Tetratrichomonas*. The generic names *Tritrichomonas* and *Pentatrichomonas* can be employed for the types with three and five flagella, or what appears to be safer is to regard the various types as varieties of one species, so that it is possible to distinguish in the human intestine *T. hominis* var.

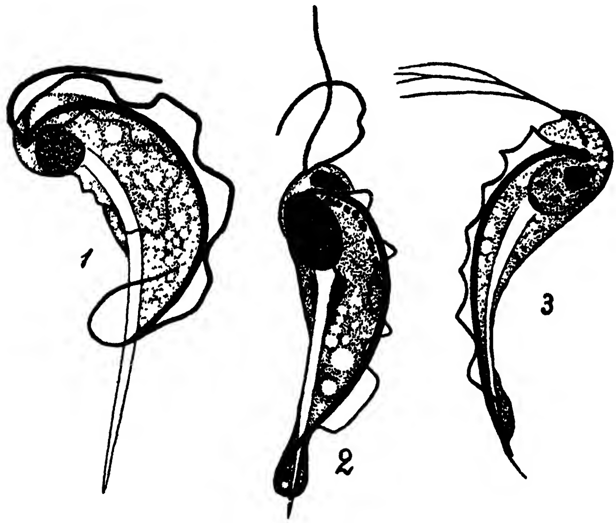


FIG. 268.—*Trichomonas trypanoides* FROM INTESTINE OF A TERMITE, *Reticulitermes lucifugus*, SHOWING ONE, TWO, AND FOUR ANTERIOR FLAGELLA ($\times$ *ca.* 2,500). (AFTER DUBOSCQ AND GRASSÉ, 1924.)

tritrichomonas, *T. hominis* var. *tetratrichomonas*, *T. hominis* var. *pentatrichomonas*. Duboscq and Grassé (1924) have shown that in the case of *T. trypanoides*, described by them from termites, there is a single thick anterior flagellum which frequently becomes divided longitudinally to give rise to two, three, or four separate flagella (Fig. 268). The commonest type in man is undoubtedly the one with four flagella. Most observers, however, fail to record the number of flagella, which, moreover, are very difficult to count. In an infection in which a certain number of flagellates have, say, four flagella, it will be found that others have a smaller number, so that to determine the prevailing type in any infection it is necessary to count the flagella on a number of flagellates, a procedure which involves

considerable expenditure of time and labour. It should be clearly understood that before a *Trichomonas* is reported as having a particular number of flagella, it is necessary to observe this number in the majority of the forms present. Some dividing forms will have a larger number of flagella, while in others it will be impossible to detect the full number. Nevertheless, by careful observation it is not difficult in the case of pure infections to determine the normal flagellum number for the form present. The writer has seen the form with four flagella on many occasions, that with five only a few times, but the one with three only once.

Derrieu and Raynaud (1914) proposed the name *Hexamastix ardin delteili* for the human form with five flagella, while Chalmers and Pekkola (1916) mistook it for a *Hexamita*, which they named *Octomitus hominis* (Fig. 289). Kofoid and Swezy (1924) employ the name *Pentatrichomonas ardin delteili* for the one with five flagella. One of the five flagella is described as a trailing flagellum longer than the others (Fig. 26).

Trichomonas elongata Steinberg, 1862.—Höffle (1850) appears to have been the first to observe *Trichomonas* in the mouth. Steinberg (1862) studied these oral flagellates and named three distinct species: *T. elongata*, *T. caudata*, and *T. flagellata*. It is evident that he was dealing with various forms of one flagellate, so that if the oral *Trichomonas* be regarded as a species distinct from that of the intestine, its proper name will be *T. elongata*, the first of those proposed by Steinberg, and not *T. buccalis*, the name suggested by Goodey and Wellings (1917). It is probable that Leeuwenhoek saw the flagellate in the tartar of his own and other people's teeth, but there are no means of identifying it unless it is assumed that *Trichomonas* is the only flagellate which can possibly occur in material taken from the mouth. That other flagellates may occur in the mouth has recently been demonstrated by Knowles and Das Gupta (1924), who have found a species of *Bodo* in this situation. Müller (1773) also noted that flagellates developed in the course of four days in water to which tartar from the teeth has been added. He named the organism *Cercaria tenax*, but there is no conclusive evidence that he was actually dealing with *Trichomonas*. It has been maintained by Goodey and Wellings (1917) that, in the oral form, the axoneme does not extend beyond the end of the membrane as a flagellum, as it does in *T. hominis* (Fig. 269). The writer has, however, found no difference in this respect between the *Trichomonas* of the mouth and that of the intestine. The writer and O'Connor (1917) noted that, in a case which constantly showed *Trichomonas* in the mouth, the flagellates never appeared in the diarrhœic stools, though they were specially looked for on many occasions. Lynch (1915*a*) could find no *Trichomonas* in the fæces of a woman who harboured *Trichomonas*, both in the mouth and vagina. The *Trichomonas* of the mouth

possesses four flagella, as noted by Goodey and Wellings (1917), and does not differ as regards size and structure from that of the intestine. Jepps (1923*a*) believes that the oral form is more actively amœboid than that of the intestine, but though it is stated that the organism agrees with the one described by Goodey and Wellings, only three flagella are figured.

Trichomonas have been noted by the writer (1920) in pus exuding from the follicles of the tonsil. Several observers, including Schmidt (1895) and Artault (1898), have seen the flagellates in sputum coughed up from the lung, while Strube (1898), Cohnheim (1903), Zabel (1904), Schmidt

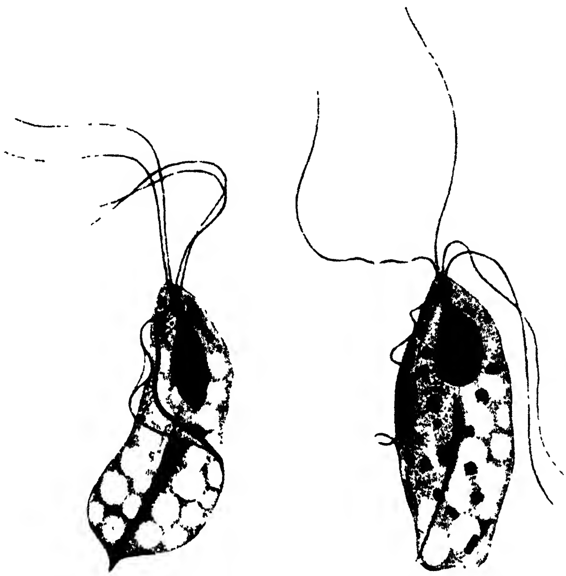


FIG. 269.—*Trichomonas elongata* FROM THE HUMAN MOUTH (× 4,100). (AFTER GOODEY AND WELLINGS, 1917.)

(1904), and Rosenfeld (1904) observed them in stomach contents in cases of carcinoma and other conditions. They have also been recorded as occurring in pleural exudate by Litten (1886) and Roos (1893). Parisot and Simonin (1921) observed the flagellates in large numbers in the expectorations of a case of gangrene of the lung. At post-mortem they were present in abundance in the gangrenous areas, but not in others. These forms are certainly identical with the oral species, though the name *T. pulmonalis* has been given to the form seen in sputum by Schmidt (1895). The invasion of the lung is comparable with the spread of spirochætes and bacteria to this organ from the mouth when conditions become favourable to their growth.

The *Trichomonas* of the mouth was maintained in culture for a short time by Lynch (1915*a*) by means of the method used by him for culture of *T. hominis*. The results with subculture were not entirely satisfactory. Ohira and Noguchi (1917) were more successful. They employ a mixture of equal parts of ascitic fluid and Ringer's solution. By making subcultures every day large numbers of organisms were obtained. The usual forms measured 10 to 15 microns by 4 to 8 microns, and possessed four flagella. Occasionally, larger forms up to 25 by 12 microns occurred. Multiple division forms, in which four, six, or eight individuals separated from the large body, were also seen (see p. 652).

Trichomonas vaginalis Donné, 1837.—This species, which was first seen by Donné (1837), is of fairly frequent occurrence in cases of vaginitis, in which the exudate has an acid reaction (Fig. 270). It has been studied especially by Blochmann (1884), Kunstler (1884), Bensen (1900), Lynch (1915*a*), Reuling (1921), and Hegner (1925). Bensen gives its measurements as varying from 18 by 6 microns to 26 by 16 microns. Some forms are narrow and elongate, while others are almost spherical. Bensen erroneously concluded that there were three anterior flagella, and also failed to note the axostyle. In a case studied by Lynch (1915*a*), *Trichomonas* was present, not only in the vagina, but also in the mouth. The oral forms are described as possessing four flagella, and what is evidently an axostyle was seen protruding from the posterior extremity. This organ was more definitely seen by Kunstler. Lynch states that the vaginal forms were the same in every respect as those in the mouth, and he concludes that the two are identical. It is interesting to note that he found no flagellates in the fæces. Reuling gives the measurements of *T. vaginalis* as 10 to 30 microns by 10 to 15 microns, and Hegner 7 to 21 microns by 6 to 18 microns. The undulating membrane, with its supporting fibre and attached axoneme, extended for only a third, or at most half, the length of the body. A definite axostyle was present, but in some cases Reuling found in its place four separate fibres (Fig. 27). Both Reuling and Hegner describe four anterior flagella.

A series measured in the living condition by the writer gave the following dimensions in microns: 29·5 by 19·2, 21·6 by 18·5, 20·0 by 16·5, 18·0 by 18·0, 18·0 by 12·6, 16·0 by 14·5, 14·5 by 9·0, 12·6 by 9·0, 11·0 by 10·0. In the infections studied, the majority of forms had four flagella (Fig. 270). Some, however, had only three, while a few which were not evidently dividing forms had five. There was a definite axostyle extending from the region of the nucleus to the posterior end, through which it projected. In many of the largest forms this structure was completely obscured. The undulating membrane extended in the larger spherical forms for only about half the length of the body, but in the smaller ones it was as long as

the body, while the attached axoneme terminated at the posterior end of the membrane or was continued for a short distance as a flagellum. In some individuals a definite cytostome could be detected, while a supporting rod at the base of the membrane was also present. Certain individuals which were evidently degenerating gave rise to structures resembling *Blastocystis* (Fig. 270, 7-9). There was very little difference in the length of the membrane in the large and small forms, the larger forms appearing

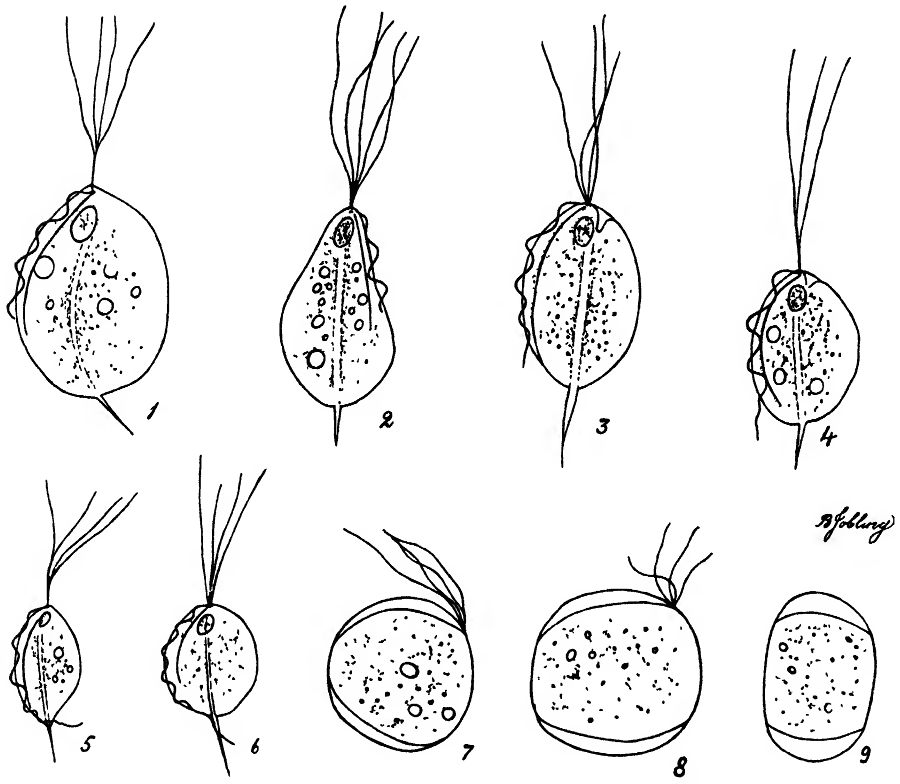


FIG. 270.—*Trichomonas vaginalis* (× 1,300). (ORIGINAL, FROM LIVING SPECIMENS.)
1-6. Typical forms. 7-9. Degenerate forms.

to have been developed from the smaller ones by overgrowth of the posterior part of the body. The smaller forms did not differ in any respect from *T. hominis* of the intestine.

From the case investigated by him, Lynch (1915*a*) obtained a culture of both the vaginal and oral *Trichomonas*, which, however, were maintained only for a short time.

A *Trichomonas* has also been found in the male urethra in cases of urethritis. It was seen in the urine by Dock (1894, 1896), Marchand (1894),

Miura (1894), and Fonseca (1916), and is not uncommon in the centrifuged deposit from urine. It is possibly the same species as *T. vaginalis.*

T. vaginalis appears to be of common occurrence in cases of vaginitis, where the exudate is acid in reaction. Thus, Hausmann (quoted by Blochmann, 1884) found it present in 30 to 40 per cent. of females examined, while Donné, its original discoverer, found it very common in France. Brumpt (1913*c*) obtained 10 per cent. positive examinations in Paris, and the writer has found it common in England. There appears to be little reason to suppose that *T. vaginalis* is in any way a pathogenic organism.

It seems quite possible that the three species of human *Trichomonas* really belong to one species, and that the differences which occur are due to variations in nutrition. The writer has studied the *Trichomonas* of the mouth, vagina, and intestine. Those of the mouth and intestine resemble one another so closely that it is impossible to differentiate them, and this is also true of the smaller forms which occur in the vagina. The large vaginal flagellates are probably overgrowth forms. Ohira and Noguchi (1917), as noted above, observed large forms of the oral *Trichomonas* in their cultures. In cultures made by the writer in Hogue's egg medium the mouth and intestinal forms were identical, and though the vaginal form was not maintained in subculture in this medium, those flagellates which remained active for some days were indistinguishable from the cultural flagellates of the mouth and intestine. Lynch (1922), who has cultivated all these forms, states that under the same conditions they are identical, and that there is no means of differentiating them. Should this prove to be the case, the name *T. vaginalis* will have priority over all others.

TRICHOMONAS IN ANIMALS.

Species of *Trichomonas* are very common parasites of the intestinal canals of animals. The cæcums and large intestines of guinea-pigs and rats, for instance, are often swarming with these flagellates, which, on account of their large size, are more easily studied than the human forms. They are common in birds, reptiles, and amphibia, and also occur in invertebrates. Many of these have been given distinctive names, but whether each host has its own species cannot be stated at present. The various species described are very uniform in character, and differ from one another chiefly in size. *T. muris* of the mouse varies in length from 3 to 20 microns at least, so that dimensions are of little value as specific characters unless they can be proved to be constant.

Trichomonas muris (Grassi, 1879).—This common flagellate of the intestine of rats and mice, and possibly other rodents, was first noted by Grassi (1879*a*), who named it *Monocercomonas muris.* Later (1881*a*) he referred

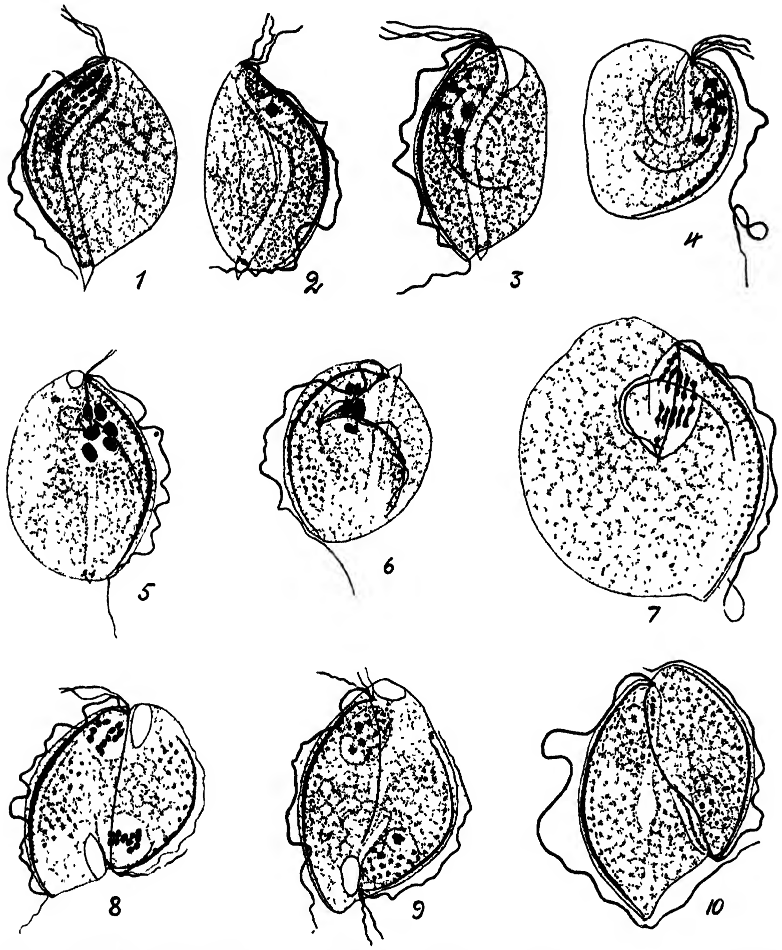


FIG. 271.—*Trichomonas muris* OF THE MOUSE, SHOWING STRUCTURE OF FLAGELLATE AND METHOD OF DIVISION (× 2,000). (AFTER WENRICH, 1921.)

1 Form showing parabasal body (pale grey streak) after fixation in Flemming's osmic acid solution.
2. Form in which parabasal body is not evident after fixation in Schaudinn's fluid.
3. Commencing division—formation of new basal fibre as fine outgrowth from blepharoplast.
4. A similar stage to that at 3—nucleus shows six chromosomes, each with indication of double nature.
5. The blepharoplasts have divided, and the daughter blepharoplasts are connected by a paradesmose; the new basal fibre and new membrane flagellum are connected with the divided-off blepharoplasts; six chromosomes with no indication of double nature.
6. Similar stage slightly more advanced, showing commencing degeneration of axostyle.
7. The six chromosomes have divided into two groups of six daughter chromosomes, which show indications of a double nature; the axostyle has disappeared.
8. Nuclear division is complete, and each daughter nucleus has six chromosomes which again appear double; the paradesmose is still present; the axostyle has disappeared.
9. The nuclei are reconstructed, and daughter axostyles (clear streaks) are growing out from the blepharoplasts.
10. Outgrowth of new axostyles complete; paradesmose still present.

to it as *Cimænomonas muris*. As it occurs in mice it was investigated by the writer (1907). It has been recorded from the field vole (*Microtus arvalis*) by Lavier (1921*b*). *T. muris* varies in length from 3 to 20 microns. It has the usual pear-shaped body, possesses three anterior flagella, a terminally protruding axostyle, and a well-developed membrane bordered by an axoneme which becomes a flagellum at the posterior end of the organism (Fig. 271). At the base of the flagella is a slit-like cytostome. In stained films the oval nucleus can be seen near the anterior end of the flagellate. It has a definite membrane, and the chromatin is distributed in the form of fine granules throughout its substance, while a central karyosome may be present. Anterior to the nucleus can be seen two groups of closely aggregated blepharoplasts. The anterior of these gives rise to the three flagella, and the one which borders the undulating membrane. From the other arises a stiff, deeply staining basal fibre, which passes down the body parallel to the base of the undulating membrane. Parallel to the basal fibre, and close to it, is a row of granules, a second row of which may also be present. The axostyle, the pointed tip of which protrudes through the body posteriorly, commences at the blepharoplasts and passes through the body. In the region of the nucleus it seems to pass between the cytostome and the nucleus, and the latter often appears to be partially embedded in it. Wenrich (1921) describes another structure which can sometimes be detected in the cytoplasm, especially after fixation in weak Flemming's solution without acetic acid. It is a sausage-shaped body lying between the nucleus and basal fibre of the undulating membrane (Fig. 271, I). It has a length of a little less than half that of the body. Wenrich considers it to be of the nature of a parabasal. A similar structure was seen by Janicki (1915) in *T. batrachorum* of the frog (Fig. 275), and by Alexeieff (1924) in *T. augusta* (Fig. 67).

The cytoplasm contains food vacuoles in which bacteria occur. Occasionally, as noted by the writer (1907), large vacuoles filled with bright refractile coccus-like bodies are seen. It was suggested that they were possibly parasitic in nature, and it is now generally recognized that they are spores of a fungus of the genus *Sphærita*, which was established by Dangeard (1886) for a similar parasite of free-living amœbæ and flagellates (see p. 252). Dangeard recognized the organism as belonging to the Chytridiaceæ, and gave it the name *S. endogena*. It was studied in free-living amœbæ by Chatton and Brodsky (1909), who gave the name *S. dangeardi* to a form in *Euglena*. What may be a distinct species was seen by the writer (1907) in *Entamœba muris* and *T. muris*, by Cragg (1919) in *E. coli*, by Dobell (1919) in *Endolimax nana*, and by Nöller (1921) in *E. coli*, *E. histolytica*, *Iodamœba bütschlii*, and *Dientamœba fragilis*. Da Cunha and Muniz (1923) gave the name *S. minor* to the form in *Trichomonas*.

Lwoff (1925), referring to the form in *E. coli* and *E. histolytica*, suggests that it is distinct from the one which parasitizes free-living amœbæ, and proposes to name it *S. normeti*. The development of the organism is a simple one. A spore enters the cytoplasm and grows into a multinucleated sphere enclosed by a membrane. It breaks up into a number of spores, which are not provided with flagella (Figs. 111, 4, and 173, 4). The spores escape from the cyst, and after entering the cytoplasm of other amœbæ or flagellates, repeat the process of growth and spore formation.

T. muris multiplies by longitudinal fission (Fig. 271). The first step in the process is the division of the two groups of blepharoplasts, so that two pairs are formed. These separate from one another, and as they do so they are seen to be connected by a fibre, the paradesmose, which may still persist even when the blepharoplasts have reached opposite sides of the body. From the anterior of the two new blepharoplasts three flagella arise, while from the posterior one a new basal fibre grows out parallel to the pre-existing one. At the same time a new membrane forms as a new axoneme grows out from the anterior blepharoplast. The new membrane, axoneme, and basal fibre gradually increase in size till they equal those already existing. Meanwhile, the nucleus has been undergoing changes. The fine chromatin granules run together to form definite chromosomes. The writer (1907) concluded there were six of these, but Kofoid and Swezy (1915*a*) give the number as five and Kuczynski (1918) as eight. Wenrich (1921) has published a clear account of the division stages of *T. muris*, and has shown that there are actually six chromosomes (Fig. 271). The nucleus becomes elongated, the nuclear membrane persisting during the whole process of nuclear division. Each chromosome then becomes constricted and divided into two, so that six pairs of chromosomes can be seen. The nucleus becomes elongated, and one set of daughter chromosomes passes to one end of the nucleus and the other set to the other end.

The nuclear membrane becomes constricted and divided. The chromosomes in each daughter nucleus now break up into finer granules, and the original type of nucleus is reproduced. During the nuclear division changes have been occurring in the axostyle. Here, again, there is a difference of opinion as to what actually happens. The writer (1907) believed that, as the blepharoplasts separate, the axostyle was divided longitudinally from before backwards. Eventually, the two daughter axostyles were united only at their posterior extremities. By this time the flagella, membrane, and other parts of the new flagellate were fully formed. The cytoplasm became elongated, and had nucleus, blepharoplasts, cytostome, and flagella at each end, while stretching between the blepharoplasts were the axostyles, united by their tips at the middle of the elongated body. The cytoplasm was then divided between the axostyles,

and two flagellates formed. They often remained united by the tips of the axostyles for some time before finally separating. In this process of division, as the blepharoplasts separate from one another, they are at

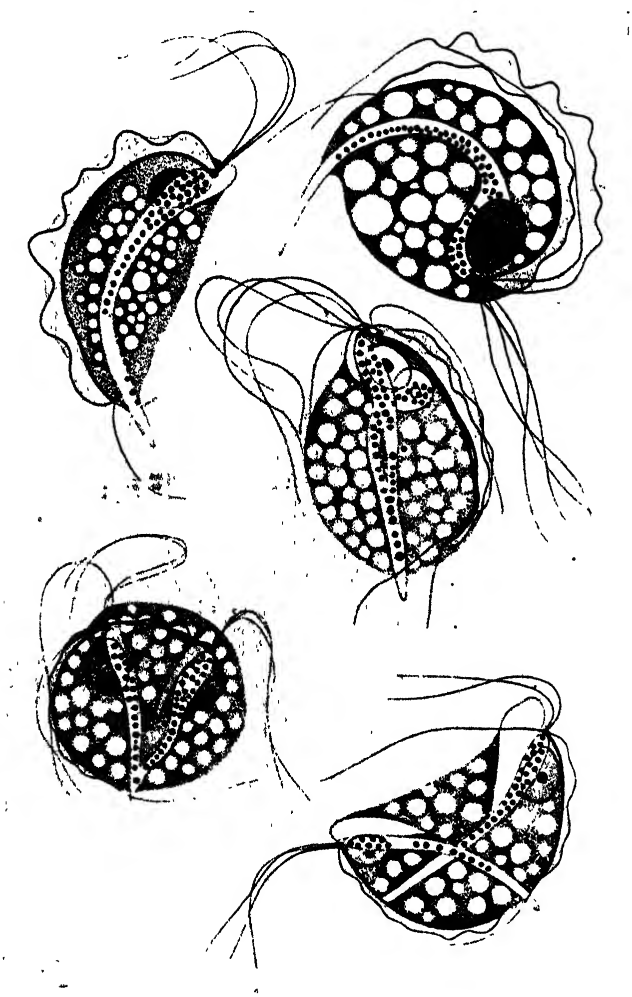


FIG. 272.—*Trichomonas augusta*, ILLUSTRATING THE STRUCTURE OF THE FLAGELLATE AND THE VIEW THAT THE AXOSTYLE SPLITS LONGITUDINALLY DURING DIVISION (× 1,450). (AFTER KOFOID AND SWEZY, 1915.)

first connected by a paradesmose, as explained above, while passing from each blepharoplast in another direction is a limb of the dividing axostyle. As the blepharoplasts take up positions at opposite poles of the elongated body of the flagellate the axostyle becomes completely divided, and as the

body of the flagellate elongates the two daughter axostyles finally occupy a straight line between the two blepharoplasts. This is the line which would be occupied by the paradesmose if it persisted, and this has given rise to the view that the axostyles are really derived from the paradesmose, and that the old axostyle had disappeared. The writer (1907) came to the conclusion that the paradesmose had disappeared before this stage, and that the structure uniting the blepharoplasts at the final stage of division was formed by the longitudinally divided axostyles. This view was supported by the observations of Kofoid and Swezy (1915*a*) on

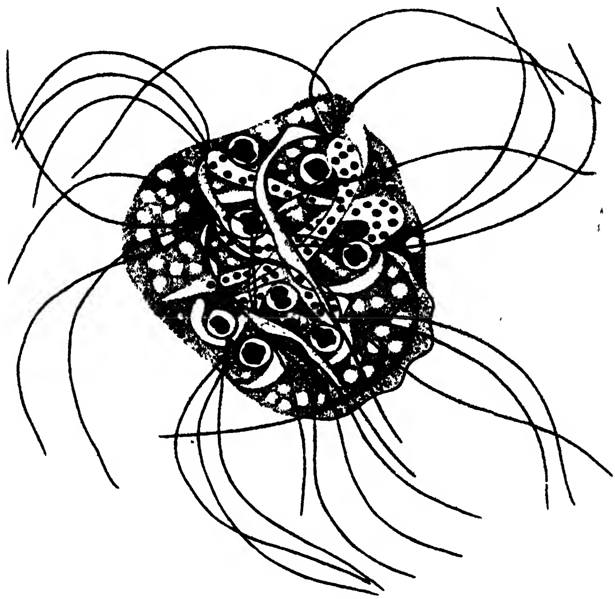


FIG. 273.—*Trichomonas augusta* FROM THE FROG, *Rana boylei:* PLASMODIUM PHASE WITH EIGHT NUCLEI AND FOUR AXOSTYLES (× 2,175). (AFTER KOFOID AND SWEZY, 1915.)

T. muris, *T. augusta*, and other species (Fig. 272). Dobell (1909) came to the conclusion, however, that in *T. batrachorum* of the frog the axostyles of the daughter flagellates arise from the paradesmose. On the other hand, Kuczynski (1914), from a study of *T. muris* and other species, maintains that neither view is correct, and that the old axostyle disappears, while new axostyles are formed as outgrowths from the blepharoplasts, and arise like the new basal fibre. Martin and Robertson (1911), from a study of *T. eberthi* of fowls, could arrive at no definite conclusions as to what happened. Wenrich (1921), from a study of *T. muris*, finds himself

in agreement with Kuczynski. The method of formation of the axostyle evidently needs reinvestigation.

Kofoid and Swezy (1915*a*) have described a remarkable process of multiple segmentation in *T. muris*, *T. augusta*, and other species (Fig. 273). By repeated divisions of the nuclei and blepharoplasts, and formation of new flagella and other structures, complex organisms are produced which may have eight nuclei and sets of organs. By multiple segmentation, eight daughter individuals are formed. These forms were not seen by the writer in a prolonged study of many mice infected with *T. muris*, nor have they been seen by other observers in those species of *Trichomonas* in which Kofoid and Swezy claim that the process occurs.

It seems probable that *T. muris* becomes encysted in spherical cysts about 6 to 8 microns in diameter. These forms were described by the writer (1907). Within the cyst can be seen the nucleus, blepharoplast, axostyle, membrane, and flagella of the flagellate. Kuczynski (1914) states that both in the case of *T. muris* and *T. caviæ* of the guinea-pig he has seen such encysted forms in which the enclosed flagellates have double sets of organs. It is often difficult to judge whether *T. muris* is encysted or not. The flagellates have a habit of becoming perfectly spherical and quiescent in passed fæces, but that such forms are not encysted can be demonstrated by warming them on the warm stage, when they will be seen to renew their activities and assume their usual form.

Wenrich (1921) believes that two species of *Trichomonas* occur in mice. The large form, *T. muris*, varies in length from 8 to 20 microns, with an average of 12·9 microns. Its nucleus in division has six chromosomes. The smaller form, which is possibly *T. parva* of Alexeieff, varies in length from 6 to 9 microns. During division its nucleus has only three chromosomes. The writer has, however, seen forms which have a length of barely 3 microns. If Wenrich's statement regarding the difference in the chromosome number is accepted, the two species must be recognized, but further information is required before his view is finally adopted.

Trichomonas caviæ Davaine, 1875.—This flagellate, first mentioned and named by Davaine (1875), is very similar to *T. muris*, and often occurs in large numbers in the cæcum and large intestine of guinea-pigs. As already remarked above, it can sometimes be seen to be invading the intestinal wall in sections of the intestine fixed immediately after death. Whether these lesions in which the flagellates occur are caused primarily by the *Trichomonas* or not has yet to be determined. Like *T. muris*, with which, indeed, it may be identical, *T. caviæ* varies in length from about 3 to 20 microns (Fig. 274). In some infections the cæcum is swarming with large forms alone, while in others every transition in size between the smallest and largest individuals can be traced.

T. caviæ becomes encysted in spherical cysts about 7 microns in diameter, as first noted by Galli-Valerio (1903). There does not appear to be any multiplication within the cyst, which is probably purely protective.

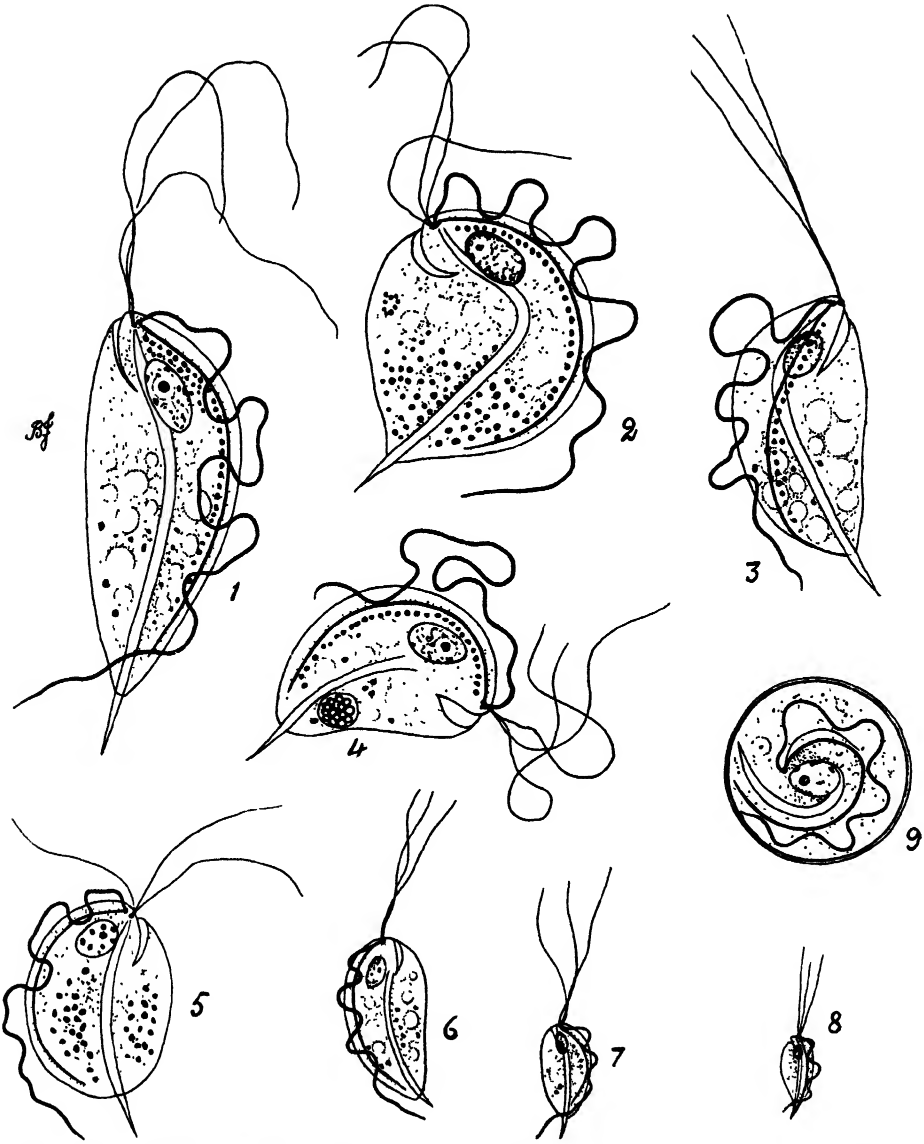


FIG. 274.—*Trichomonas caviæ* FROM LARGE INTESTINE OF GUINEA-PIG (×3,000). (ORIGINAL.)

1-8. Flagellates from one preparation, showing great variation in size.
9. Encysted form.

The flagellate has been cultivated by Chatton (1920). He employed a medium consisting of ordinary bouillon, to which had been added 1 c.c. of blood to every 10 c.c. of bouillon. In this, *T. caviæ* grew in association with numerous bacteria. By subculture every three or four weeks the cultures were maintained for a year, when they were lost owing to accidental contamination with fungi. The culture apparently grew at any temperature between 20° and 37° C., but they survived longer at the lower temperature, when multiplication of the bacteria and flagellates was less rapid than at higher temperatures. Though the cultures were started from typical *Trichomonas* with undulating membrane, the flagellates assumed the *Eutrichomastix* form in culture when the axoneme bordering the membrane became a free flagellum. In attempts to rid the cultures of bacteria, guinea-pigs were inoculated intraperitoneally with culture. Six hours after, when the peritoneum was examined, the flagellates had assumed the *Trichomonas* form again. Chatton believes that *Eutrichomastix caviæ*, which in natural infections is very frequently found along with the *T. caviæ*, is merely a form of this flagellate which it assumes in media of low density.

Faust (1921*a*) has stated that the *Trichomonas* which occurs in guinea-pigs in Pekin differs from those described from this animal elsewhere. The size of the organism is given as 8 to 14 microns by 6·5 to 10 microns. The protruding portion of the axostyle is said to be two-thirds the body length. There are three anterior flagella, which have a length over half that of the body and a long posterior flagellum. On account of the supposed difference from *T. caviæ*, Faust proposes to call this form *T. flagelliphora*. From the plate accompanying his description, which the author says depicts characteristic specimens, the writer can find no evidence that he is dealing with a species distinct from the ordinary form which is common in guinea-pigs in other localities.

Other Species of Trichomonas.

A large number of other species of *Trichomonas* have been described, and these have been studied especially by Dobell (1909), Alexeieff (1909-1911), Kuczynski (1914), and Kofoid and Swezy (1915). They occur in a variety of hosts, as summarized below, and many specific names have been given, but it is clear that in most cases the evidence necessary for the establishment of new species is wanting.

Mammals.—*T. suis* Gruby and Delafond (1843) (stomach of pig); *T. tatusi* Fonseca, 1915, three free flagella (*Tatus novemcinctus*, armadillo); *T. ruminantium* Braune, 1913, three free flagella (rumen of cattle). Fantham (1920) records this form from the reticulum of the sheep and ox, and (1921) gives the name *T. equi* to one in the horse. *T. chagasi* Haselmann and Fonseca, 1918, three free flagella (*Cerodon rupestris*); *T. felis* Da Cunha and Muniz, 1922, four free flagella (cat); Brumpt

(1925) (cat and dog). Brumpt (1909*a*) noted a form in *Macacus sinicus*. Fantham (1925) records *T. mystromyis* from the white-tailed rat (*Mystromys albicaudatus*).

Birds.—*T. eberthi* Martin and Robertson, 1911, three free flagella; and *T. gallinarum* Martin and Robertson, 1911, four free flagella (cæcum of fowls). *T. columbæ* Rivolta, 1878 (pigeons), and *T. columbarum* Prowazek and Aragão (1909), are possibly the same as *T. columbæ*. Ratz (1913*a*) observed a *Trichomonas* in the liver of a pigeon, while Kotlan (1923) described *Trichomonas eberthi* and a new species, *Tetratrichomonas anatis*, from the cæcum of ducks.

Lizards.—*T. lacertæ* Prowazek, 1904, three free flagella (*Lacerta* sp. and other lizards); *T. mabuiæ* Dobell, 1910, three free flagella (*Mabuia carinata*); *T.* sp. Dobell, 1910, three free flagella (*Hemidactylus leschenaulti*); *T.* sp. Wenyon, 1921, three free flagella (*Agama stellio* and *Lacerta agilis*); *T.* sp. Franchini, 1921 (*Lacerta ocellata*).

Snakes.—The writer has seen and cultivated a form with three free flagella from *Python molurus* of India.

Tortoises.—*T. brumpti* Alexeieff, 1912, four free flagella (*Nicoris trijuga*). It has been seen by the writer in other tortoises (*Testudo radiata*, *T. calcarata*, and *T. argentina*).

Crocodiles.—A form identified as *T. prowazeki* was seen by Parisi (1910) in *Crocodilus palustris*.

Amphibia.—*T. batrachorum* Perty, 1852, three free flagella (frogs, toads, and newts, etc.) (Fig. 275); *T. augusta* Alexeieff, 1911, three free flagella (frogs, toads, and newts, etc.); *T. prowazeki* Alexeieff, 1909, four free flagella (*Salamandra maculosa*, *Triton cristatus*, *Alytes obstricans*); *T. tritonis* Alexeieff, 1911, three free flagella (newts); *T. mirabilis* Kuczynski, 1918, three free flagella (*Bufo* sp. of the Congo); *Tetratrichomonas batrachorum* Escomel, 1925, four flagella (*Telmatobius gebski*, South America). *Exechlyga acuminata* Stokes, 1884, is probably *T. batrachorum*.

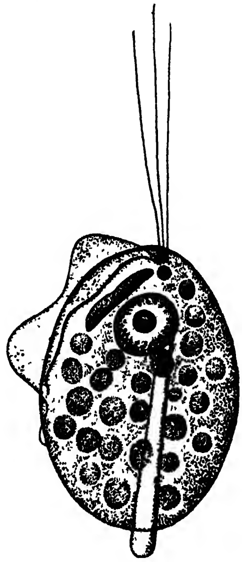


FIG. 275.—*Trichomonas batrachorum* OF THE FROG, SHOWING PARABASAL BODY AFTER FIXATION IN HERMANN'S FLUID (× 2,400). (AFTER JANICKI, 1915.)

The axostyle is abnormal in not being pointed at its posterior end.

Fish.—*T. legeri* Alexeieff, 1910, three free flagella (*Box boops*); *T. prowazeki* Alexeieff, 1910, four free flagella (*Box salpa*); *T.* sp. Fantham, 1919 (*Mugil capito*).

Leeches.—*T. sanguisugæ* Alexeieff, 1911, three free flagella (*Hæmopis sanguisuga*); *T. granulosa* Alexeieff, 1911, three free flagella (*Hæmopis sanguisuga*); *T. ninæ kohl-yakimowi* Yakimoff, 1917 (*Luminatis turkestanensis*).

Molluscs.—*T. limacis* Dujardin, 1841 (land snail, *Limax agrestis*).

Termites.—*T. termitis* Dogiel, 1916, four free flagella (*Rhinotermes* sp.); *T. macrostoma* Dogiel, 1916, four free flagella (*Hodotermes mossambicus*); *T. dogieli* Duboscq and Grassé, 1923; three free flagella (*Calotermes flavicollis*); *T. trypanoides* Duboscq and Grassé, 1924, four free flagella (*Reticulitermes lucifugus*); *T. termopsidis* Cleveland, 1925, four free flagella (*Termopsis nevadensis*).

Invasion of the Blood-Stream by Trichomonas.

The observation of Pentimalli (1923) of *Trichomonas* in the human blood-stream has been mentioned above (p. 653). Lanfranchi (1908)

discovered a *Trichomonas* in the blood of a pigeon. He claimed to have inoculated it to rabbits and guinea-pigs. Martoglio (1917) discovered a similar form which had four free flagella in the blood of fowls in Eritrea, and proposed to place it in a new genus as *Hæmotrichomonas gallinarum*. He also places in this genus as *H. ophidium* the *Trichomonas* discovered by Plimmer (1912*a*) in the blood of snakes which had died in the Zoological Gardens. Lanfranchi (1917) again refers to the form previously described by him, and places it in Martoglio's genus as *H. columbæ*. These forms, which occur in the blood, are almost certainly the result of invasion of the vessels by intestinal flagellates, so that there is no justification for the genus *Hæmotrichomonas*, as indeed Sangiorgi (1922), who saw a *Trichomonas* in the heart blood of a dead mouse, has pointed out. For some reason which is not quite clear he believes that the flagellates seen by Lanfranchi and Martoglio in the blood of the fowl and pigeon were not *Trichomonas*, but *Toxoplasma*. As pointed out by Plimmer (1912), the intestinal *Trichomonas* of amphibia are liable to invade the blood-stream shortly before death. As noted above, the writer (1920) has seen *T. hominis* in the tissues of the intestinal mucosa of human beings (Fig. 267).

Genus: **Gigantomonas** Dogiel, 1916.

This genus was established by Dogiel (1916) for a flagellate of the intestine of the termite, *Hodotermes mossambicus*. The chief characters are the size and the fact that one of the anterior flagella is thicker and longer than the others.

Gigantomonas herculea Dogiel, 1916.—This is the only representative of the genus. It measures from 60 to 75 microns in length and 30 to 35 microns in breadth. In structure it resembles a *Trichomonas*. It seems possible that the flagellate represents an overgrown form of *T. macrostoma*, which Dogiel found in the same host.

Genus: **Ditrichomonas** Cutler, 1919.

This is a genus which was founded by Cutler (1919) to include a flagellate of termites which has essentially the same structure as *Trichomonas* (Fig. 276). The single species, *D. termitis*, possesses only two anterior flagella. It has two blepharoplasts, a nucleus, axostyle, and a basal fibre running along the line of attachment of the undulating membrane, to which the backwardly directed flagellum is attached. One of the blepharoplasts, which Cutler terms the membrane granule, gives rise to the basal fibre and the axoneme of the posterior flagellum. The other gives origin to the axonemes of the two anterior flagella and the axostyle, as well as a rod-shaped body called the parabasal. The latter structure may be half the length of the body, and appears to be homologous with the

parabasal described by Janicki (1915) in *Devescovina striata* (Fig. 32) and *T. batrachorum* (Fig. 275). Similar though smaller parabasal bodies have been described in species of *Trichomonas*. Thus, they were seen in *T. augusta* (Fig. 67) by Alexeieff (1911*h*) and Kuczynski (1914). The latter observer (1919) found the parabasal of constant occurrence in *T. mirabilis*, which also possessed the basal fibre, so that the view of Kofoid and Swezy (1915*a*) that the basal fibre of *Trichomonas* is homologous with the parabasal of other flagellates is untenable.

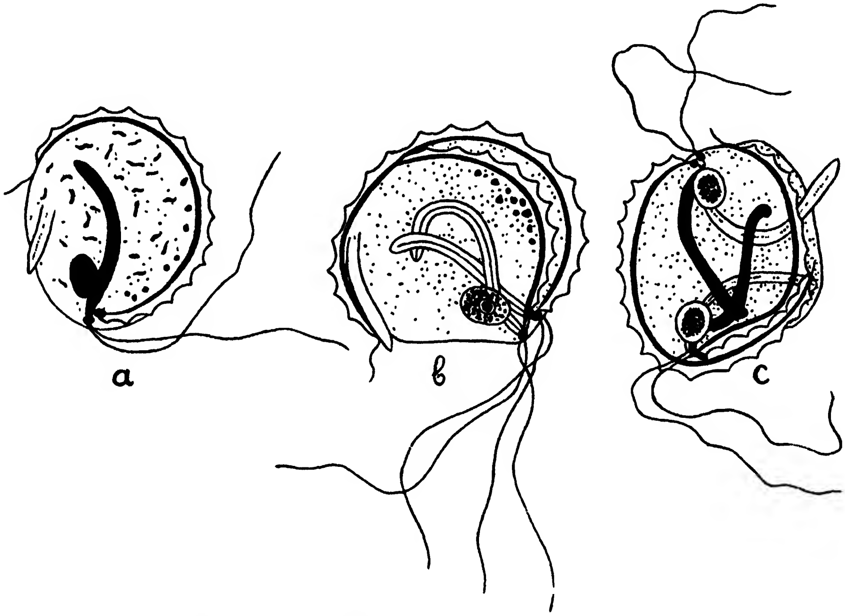


FIG. 276.—*Ditrichomonas termitis* (× 940). (AFTER CUTLER, 1919.)

a. Usual type of flagellate, showing the deeply staining parabasal body.
b. Dividing form, showing two basal fibres and membranes and new axostyles developing.
c. Later division stage, showing duplication of all the structures.

Duboscq and Grassé (1924) describe as *T. trypanoides* a flagellate of termites which has a single thick anterior flagellum. In certain individuals it is represented by two, three, or four finer flagella (Fig. 268). They include Cutler's flagellate in the genus *Trichomonas*, and propose for it the name *Trichomonas immsi*, as the name *T. termitis* was employed by Dogiel (1916) for another form in white ants.

Genus: **Eutrichomastix** Kofoid and Swezy, 1915.

This genus includes flagellates, which resemble *Trichomonas* except for the absence of an undulating membrane, the posterior flagellum of *Trichomonas* being represented by a trailing flagellum (Fig. 265, D). They

have generally been known by the generic name *Trichomastix*, but owing to the fact that Vollenhoevan had previously proposed this name for an insect, Kofoid and Swezy (1915*a*) introduced the name *Eutrichomastix*. It seems probable that, in some cases at least, the *Eutrichomastix* forms are merely *Trichomonas* in which the posterior flagellum has become free. Chatton (1920), as noted above, found that in cultures the *Trichomonas* of the guinea-pig might assume either form. Reichenow (1918, 1920*b*) noted that occasionally in lizards (*Lacerta muralis* and *L. viridis*) the blood-stream was invaded by *Eutrichomastix* from the intestine. In one case in which a lizard had died of such an infection, at the time of death the only forms present in the blood were of the *Eutrichomastix* type. On the next day, however, in addition to these there were other flagellates of the *Trichomonas* type present. Reichenow considers it possible that the latter had been derived from the former, and that the two types may be stages of one organism. In favour of this view is the well-known fact that where flagellates of the *Trichomonas* type occur, very frequently others of the *Eutrichomastix* form are present at the same time. Thus, Dobell (1909) noted that *T. batrachorum* was often associated in the frog's intestine with *E. batrachorum*, and a similar association was noted by Prowazek (1904*a*) in the case of lizards, and by Martin and Robertson (1911) in fowls. On the other hand, it appears that sometimes the flagellates are found in the *Eutrichomastix* form when *Trichomonas* is absent, as in the case of *E. serpentis* seen in a snake by Dobell (1907*a*). The writer has cultivated a *Trichomonas* of the tortoise (*Testudo radiata*), the python (*Python molurus*), and the frog, and in these cases there was no tendency for the flagellates to assume the *Eutrichomastix* form. For the present, therefore, it seems best to regard the flagellates as belonging to distinct genera.

The flagellates of the genus *Eutrichomastix* have the same structure as those of the genus *Trichomonas*, except that all the flagella, which are four in number, are free, there being no undulating membrane. One of the four flagella usually functions as a trailing flagellum.

It is unnecessary to give a detailed description of these flagellates, which in their life-history and structure correspond very closely with the various species of *Trichomonas*.

Haughwout and Horrilleno (1920) state that they saw a flagellate of the *Eutrichomastix* type in a human stool in Manila. They refer to it as *Eutrichomastix* sp. As only a single flagellate was seen, it is possible that they were dealing with an altered *Trichomonas*.

E. lacertæ was described by Prowazek from the intestine of species of *Lacerta*. What is probably the same form occurs also in other lizards, as noted by the writer (1921) in the case of *L. agilis* and *Agama stellio*.

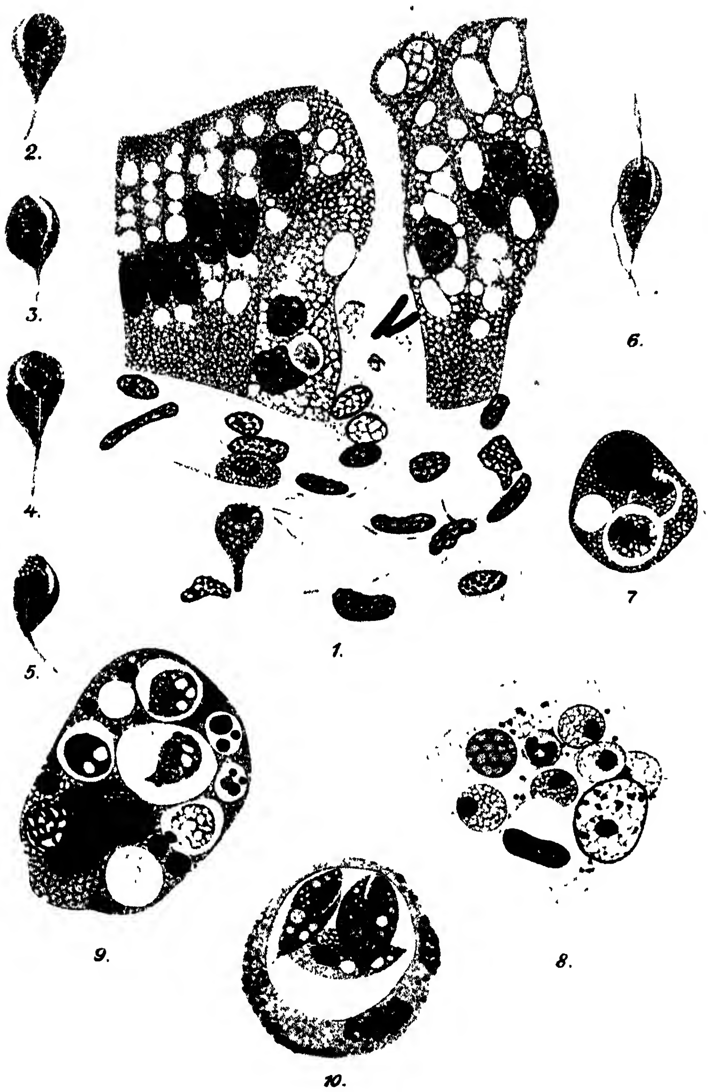


FIG. 277.—*Eutrichomastix lacertæ* IN THE LIZARD AND THE MITE (× *ca.* 1,300). (AFTER REICHENOW, 1920.)

1. Section of intestine of lizard (*Psammodromus hispanicus*), showing wound of epithelium into which a flagellate and bacteria have penetrated.
2 6. Flagellates from the blood of the lizard (*Lacerta muralis*).
7. Lymphocyte in the blood of the lizard with two ingested flagellates.
8. Large mononuclear cell from blood of lizard with ingested flagellates.
9. Large cell from body cavity of lizard with ingested flagellates.
10. Intestinal epithelial cell of the mite (*Liponyssus saurarum*) with included flagellates.

E. batrachorum was described in detail by Dobell (1909) and *E. serpentis* by Kofoid and Swezy (1915*a*). In the latter case, multiplication by binary fission, as also by multiple segmentation with the production of eight daughter individuals, is described, as noted by these authors in the case of species of *Trichomonas* (p. 666). Dobell (1909) described the encysted forms of *E. batrachorum* and *T. batrachorum* as small ovoid bodies measuring 6·5 by 5 microns. They bear a striking resemblance to the cysts of species of *Embadomonas* (Fig. 255, 14-19). The writer on one occasion obtained a culture of an *Embadomonas* from the rectum of the common English frog. The encysted forms corresponded very closely with those described by Dobell, so that it seems very probable that the supposed cysts of *E. batrachorum* and *T. batrachorum* actually belonged to undetected *Embadomonas*. Working with *E. lacertæ*, Reichenow (1918, 1920*b*) noted that the flagellate sometimes invaded the intestinal wall, body cavity, and even the blood-stream of the lizards (*Lacerta*), and that the mites (*Liponyssus saurarum*) which suck their blood become infected with the same flagellate (Figs. 277, 458). In mites which have a second feed of blood, the flagellates multiply rapidly and increase in size. They occur in numbers in large vacuoles in the lining cells of the intestine. It was demonstrated by Reichenow that the mites can remain infected for at least thirteen days, and he succeeded in infecting a newly hatched *Lacerta muralis* by feeding it on infected mites. In this connection it is interesting to note that Chatton (1918*a*) obtained a culture of a species of *Eutrichomastix* from the heart blood of the North African gecko, *Tarentola mauritanica*. These cultures, which contained bacteria in addition to flagellates, were maintained indefinitely in subculture.

As in the case of the genus *Trichomonas*, numbers of species of *Eutrichomastix* have been given names. *Trichomastix hominis*, described by Chatterjee (1917*a*), is probably a small form of *Chilomastix mesnili* (see p. 306), and it seems probable that some of the forms ascribed to the genus really belong to *Trichomonas*, the posterior flagellum having become detached from the undulating membrane.

E. ruminantium (Braune, 1913) occurs in the rumen of cattle, while in fowls is found *E. gallinarum* (Martin and Robertson, 1911). Kotlan (1923) has described this species from ducks, while Da Cunha and Muniz (1925) have named three species from Brazilian birds. *E. caviæ* (Grassi, 1881) is parasitic in the cæcum of the guinea-pig. Yakimoff, Wassilewsky, Korniloff, and Zwietkoff (1921) give the name *E. caviæ* var. *rossica* to a form seen by them in the guinea-pig, and which is undoubtedly identical with *E. caviæ*. Fonseca (1916) records *E. caviæ* from the wild guinea-pig (*Cavia aperea*) and the aguti (*Dasyprocta aguti*) of Brazil. In reptiles there are several named species, all of which may belong to the form *E. lacertæ* (Bütschli, 1844), which was redescribed by Prowazek (1904*a*) from species of *Lacerta* and by Franchini (1921*a*) from *Lacerta ocellata*. *E. viperæ* (Léger, 1904) occurs in *Vipera aspis* and *E. serpentis* (Dobell, 1907) in *Boa constrictor*. *E. mabuiæ* (Dobell, 1910) occurs in the Ceylon lizards, *Hemidactylus leschenaulti* and *Mabuia carinata*, and *E. saurii* (Fonseca, 1917) in a Brazilian lizard, *Amphisbæna* sp. *E. batrachorum*

(Dobell, 1909) occurs in frogs, and probably other amphibia. From fish there have been recorded *E. motellæ* (Alexeieff, 1910) from *Motella tricerrata* and *E. salpæ* (Alexeieff, 1910) from *Box salpa.* In invertebrates are found *E. trichopteræ* (Mackinnon, 1910) from trichopteran larvæ. It was recorded also by Mackinnon (1915) from tipulid larvæ (Fig. 278). Mackinnon (1913) discovered a flagellate in tipulid larvæ which differed from *Eutrichomastix trichopteræ,* which was also present, in that it possessed four, instead of three, anterior flagella in addition to the trailing flagellum. For this reason it was placed in a new genus as *Tetratrichomastix parisii.* In a later communication Mackinnon (1915) described spherical cysts 4 to 5 microns in diameter. The nucleus of the single flagellate within the cyst divided once to form two nuclei. These cysts belonged either to *T. parisii* or *E. trichopteræ.*

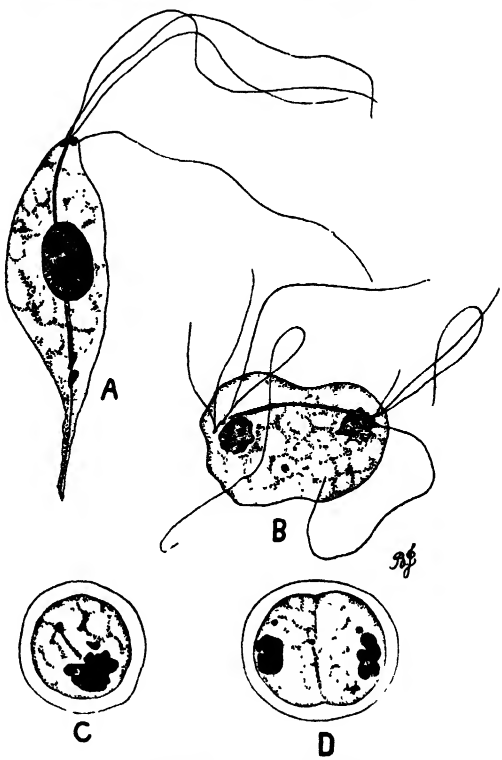


FIG. 278.—*Eutrichomastix trichopteræ* FROM INTESTINE OF *Trichoptera* LARVÆ (× *ca.* 2,600). (AFTER MACKINNON, 1910.)

A. Flagellate showing four anterior flagella, one of which is a trailing flagellum; nucleus is somewhat farther back than usual; axostyle is shown, but not the cytostome, which is sometimes clearly visible. B. Dividing form. C. Encysted form. D. Division within the cyst.

Genus: Janickiella Duboscq and Grassé, 1923.

Duboscq and Grassé (1923) created a new genus, *Janickiella,* for a flagellate (*J. grassii*) which they found in the intestine of the termite, *Calotermes flavicollis.* In many respects it resembles members of the genus *Eutrichomastix* (Fig. 279, 3). It is ovoid in shape, with a cytostome and long protruding axostyle. In front of the anteriorly situated nucleus are two blepharoplasts. One of these is large and gives origin to a long, thick, trailing flagellum and a rod-like parabasal. The other is small, and from it arise the axonemes of three fine anteriorly directed flagella and two rows of granules. In addition to this flagellate, the termites harboured other forms. Two of these were very small flagellates which resembled *Trimitus* with two anterior flagella or *Tricercomonas* with three anterior flagella (Fig. 279, 1-2). Duboscq and Grassé (1924*a*), as a result of further observations, have reached the conclusion that the small flagellates are young stages of the *Eutrichomastix* form, which is itself merely a young form of

Trichomonas dogieli (Fig. 279, 4). Is it further suggested that other flagellates, such as *Jœnia*, may enter into the life-cycle of *Janickiella grassii*, and that the flagellates belonging to the genera *Enteromonas* and *Tricercomonas* may be merely young forms of others. It has been pointed out above that *E. hominis* is probably a young form of *Chilomastix mesnili* (see p. 307).

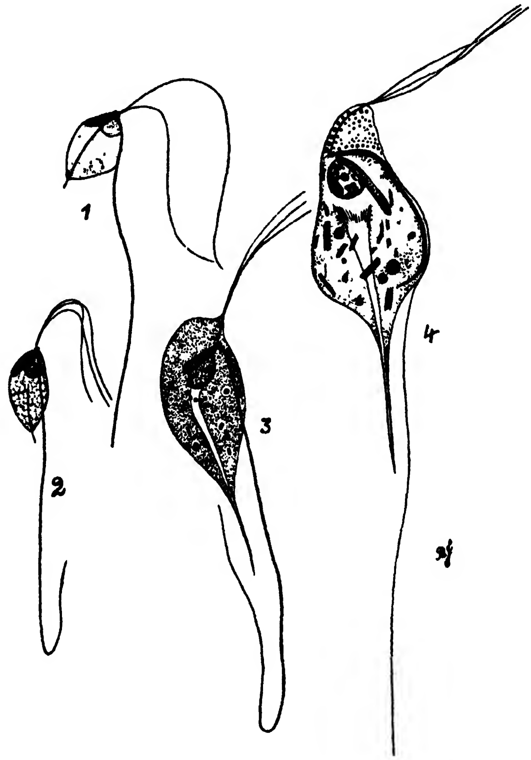


FIG. 279.—FLAGELLATES FROM INTESTINE OF THE TERMITE, *Calotermes flavicollis*, ILLUSTRATING THE DEVELOPMENT OF *Janickiella grassii* (× *ca.* 2,000). (AFTER DUBOSCQ AND GRASSÉ, 1924.)

1. "Trimitus" stage with two anterior flagella.
2. "Tricercomonas" stage with three anterior flagella.
3. "Eutrichomastix" stage.
4. "Trichomonas" stage.

Genus: Trichomitus Swezy, 1915.

This genus was founded by Swezy (1915*a*) for a flagellate from amphibians. It resembles a member of the genus *Trichomonas* with three flagella, but differs in the absence of an axostyle. It was named *Trichomitus parvus*. Later Kofoid and Swezy (1919) placed in this genus as *T. termitidis* a structurally similar but much larger flagellate found in the termite, *Termopsis angusticollis*, of California. It varies in length from 75 to 150 microns. An elaborate system of fibres, called the neuromotor

system, is described in connection with the blepharoplasts and nucleus. In addition to multiplication by binary fission, a process of multiple fission is said to occur. As during division the nucleus behaves differently from that of *Trichomitus parvus*, it is suggested that *T. termitidis* be regarded as belonging to a sub-genus, *Trichomitopsis*.

In connection with this genus, it has to be remembered that the detection of an axostyle in *Trichomonas* is not always a simple matter. In any preparation containing large numbers of *Trichomonas*, a number of forms always occur in which an axostyle is not visible. Furthermore, in the large overgrown forms of *T. vaginalis*, the axostyle is frequently quite obscured, so that it seems possible that the forms included in the genus *Trichomitus* may in reality belong to the genus *Trichomonas*.

Genus: Devescovina Foa, 1905.

This is a genus which was established by Foa (1905) to include certain flagellates which occur in the intestine of termites. The genus is undoubtedly related to *Eutrichomastix*. *D. striata* has been studied by Janicki (1911). There are four flagella, three of which are directed forwards, while one, which is much longer than the others, acts as a trailing flagellum (Fig. 32). There is a blepharoplast from which the flagella arise, and behind it is the nucleus, which appears to be embedded in the axostyle. In relation to the nucleus and coiled round the anterior part of the axostyle is an elongate deeply staining body, the parabasal. It is with this structure that Kofoid and Swezy (1915*a*) homologize the basal fibre of *Trichomonas*.

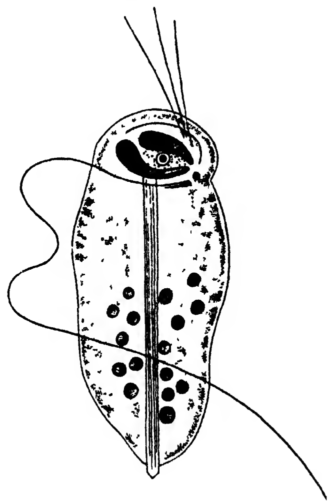


FIG. 280.—*Foaina gracilis* FROM INTESTINE OF THE TERMITE, *Calotermes castaneus* (× 1,825). (AFTER JANICKI, 1915.)

Genus: Foaina Janicki, 1915.

This genus was created by Janicki (1915) to include a flagellate of termites, which resembles *Devescovina* in many respects (Fig. 280). In place of the long coiled parabasal there are two small parabasals.

Genus: Retortamonas Grassi, 1879.

Grassi (1879*a*) created three new genera: *Monocercomonas*, *Retortamonas*, and *Schedoacercomonas*. In the first genus he included intestinal flagellates of man, guinea-pig, snake, frog, mouse, and lizard. It is probable that all these were *Trichomonas*, and that *Monocercomonas* is a synonym of *Trichomonas*. The name

has, however, been generally used for another group of flagellates owing to the fact that Grassi (1881*a*) included in the genus a form which he called *Monocercomonas insectorum*, a name which he regarded as including two flagellates previously named by him (1879*a*) *Schedoacercomonas gryllotalpæ* and *S. melolonthæ*. Neither of these is a *Trichomonas*, so that Grassi (1881*a*) was quite wrong in placing them in his genus *Monocercomonas*, which included a number of undoubted *Trichomonas*. In 1879, however, he had given the name *Retortamonas gryllotalpæ* to a flagellate of the mole cricket, and as this name was placed before *Schedoacercomonas gryllotalpæ* and *S. melolonthæ*, both of which appear to belong to the same genus, the correct generic name for these flagellates is *Retortamonas*, and not *Monocercomonas*. The question was still further complicated by the fact that Grassi (1881*a*), without any apparent reason, altered the name *Retortamonas gryllotalpæ* to *Plagiomonas gryllotalpæ*, which is therefore merely a synonym.

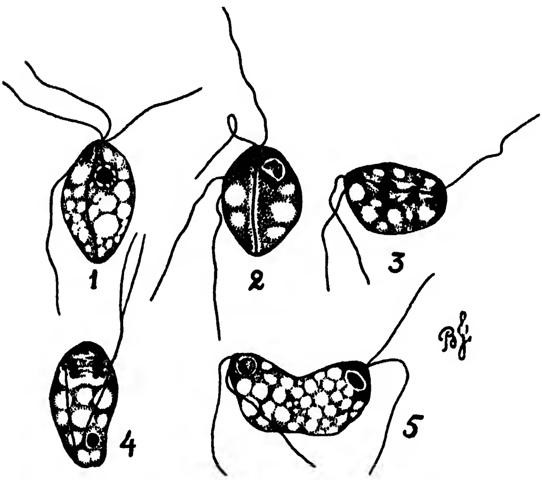


FIG. 281.—*Retortamonas orthopterorum* (× 3,800). (AFTER BĚLAŘ, 1916.)

1-2. Flagellates showing four flagella (one a trailing flagellum), axostyle, nucleus, and blepharoplast.
3-5. Division stages.

The flagellates of the genus *Retortamonas* are closely allied to *Eutrichomastix*. There are four flagella, one of which is a trailing flagellum. In the place of the typical axostyle of *Eutrichomastix*, there is a fibre which stains deeply. In many of the flagellates, however, such a fibre cannot be distinguished, and they resemble Monadidæ with four flagella (see p. 308). The first forms to be described were *Retortamonas gryllotalpæ* Grassi, 1879 (syns. *Schedoacercomonas gryllotalpæ* Grassi, 1879; *Monocercomonas insectorum* Grassi, 1881, *pp.*.; *Plagiomonas gryllotalpæ* Grassi, 1881), of the mole cricket, *Gryllotalpa* sp., and *R. melolonthæ* Grassi, 1897 (syns. *S. melolonthæ* Grassi, 1879; *M. insectorum* Grassi, 1881, *p.p.*) of the cockchafer, *Melolontha vulgaris*. Parisi (1910) described as *Trichomastix orthopterorum* a similar form from the cockroach, while Jollos (1911) gave the name *Monocercomonas cetoniæ* to one from larvæ of *Cetonia* sp. Hamburger (1912) also studied this flagellate. Mackinnon (1912) observed a form in tipulid larvæ, while França (1913) described forms from *Oryctes nasicornis*, *O. grypus*, and *Phyllognatus silenus*. Bělař (1916) gave a detailed account of the structure and division of *R. orthopterorum*. The organism is pear-shaped as a rule, and measures 3 to 6 microns in length. There is no cytostome (Fig. 281). Four flagella arise from the blepharo-

plast near the nucleus at the anterior end of the body. One of the flagella functions as a trailing flagellum. Arising in the blepharoplast and passing through the body to its posterior end is an axostyle. This structure cannot, however, be distinguished in all the forms. Furthermore, the axostyle appears to differ from the corresponding structure in *Trichomonas* and *Eutrichomastix* in that it stains deeply with iron hæmatoxylin. It is possibly not an axostyle at all in the strict meaning of the term.

Very frequently *Retortamonas* occurs in association with *Polymastix*, which differs chiefly in its peculiarly ridged periplast. Mackinnon (1912) noted that *Polymastix* not infrequently cast its periplast, with the result that flagellates of the *Retortamonas* type resulted.

Genus: Protrichomonas Alexeieff, 1911.

Very closely allied to *Retortamonas* is the genus *Protrichomonas*, which was founded by Alexeieff (1911*h*) for a flagellate which he discovered

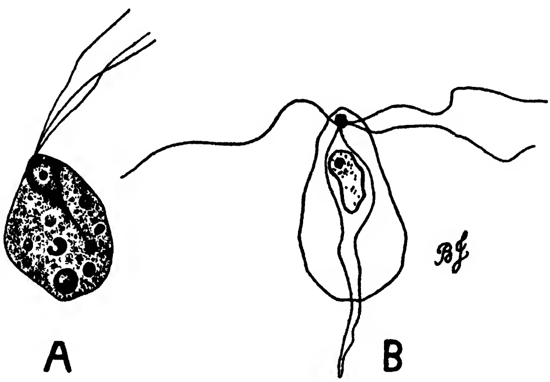


FIG. 282.—(A) *Protrichomonas legeri* (ALEXEIEFF, 1910), FROM ŒSOPHAGUS OF MARINE FISH, *Box salpa* (× 1,500). (B) *Protrichomonas anatis* KOTLÁN, 1923, IN RECTUM OF DUCK (× 2,000). (A, AFTER ALEXEIEFF, 1910; B, AFTER KOTLÁN, 1923.)

(1910) in the œsophagus of the marine fish, *Box boops* (Fig. 282). Alexeieff (1910) noted that it had three anterior flagella of equal length arising from a blepharoplast in front of the nucleus. A structure like an axostyle passed backwards through the body from the blepharoplast. He named the parasite provisionally *Trichomonas* (?) *legeri*, in spite of the fact that there was no undulating membrane. Later (1911*h*) he came to the conclusion that it did not belong to the genus *Trichomonas*, and placed it in a new genus, *Protrichomonas*.

Kotlán (1923) ascribed to this genus, under the name *P. anatis*, a flagellate which he found in the intestine of ducks and other aquatic birds

(*Nyroca ferruginea* and *Fulica atra*). The flagellate has an ovoid body measuring 10 to 16 microns by 4 to 6 microns. There are three anterior flagella as long as, or longer than, the body. They arise from an anteriorly placed blepharoplast. The nucleus is situated near the centre of the body. Arising from the blepharoplast, and passing backwards through the cytoplasm, are two fibres. They pass one on each side of the nucleus, and then run close together to the posterior extremity of the body, through which they protrude as a pointed body. From the figures, these two fibres appear as if they might be the margins of an axostyle.

FIG. 283.—*Polymastix melolonthæ* FROM GUT OF INSECT LARVÆ (× 4,000). (AFTER MACKINNON, 1913.)

1. Ordinary type of flagellate. 2. Dividing form.

Genus: **Polymastix** Bütschli, 1884.

Bütschli established this genus for a flagellate to which Grassi (1881*a*) has referred as *Trichomonas melolonthæ* from the intestine of the larva of the cockchafer (*Melolontha*). Similar forms were discovered by Hamburger (1911) in larvæ of *Cetonia* sp., Mackinnon (1912, 1913) in larvæ of *Tipula* sp., and França (1913) in larvæ of *Oryctes nasicornis*. The form studied by

Mackinnon appears to be the same as *P. melolonthæ* of the cockchafer (Fig. 283). The body is pear-shaped, with a rounded anterior and pointed posterior end, which may be forked or otherwise deformed. There are four flagella arising in pairs from two blepharoplasts at the anterior end of the flagellate. Between the blepharoplasts, according to Mackinnon, there is a cytostome. The nucleus lies just behind the blepharoplasts, and it is spherical or pear-shaped. It contains a large karyosome. A characteristic feature of the flagellate is the presence of a definite rigid periplast, which is raised into ridges or folds which run in a more or less longitudinal direction. An axostyle is present, but is not always well developed. The flagellate multiplies in a somewhat curious manner. The karyosome becomes dumb-bell-shaped and then divided, and this is followed by division of the nucleus. One nucleus, together with one of the blepharoplasts and its two flagella, and part of certain granules which lie just anterior to the nucleus, become gradually transferred to the posterior end of the organism which elongates. The body is then divided by constriction across the middle. This form is of interest in that it shows features which characterize some of the highly complicated forms included in the order Hypermastigida, such as *Lophomonas blattarum*, a flagellate which occurs in the intestine of the cockroach (Fig. 286). The mode of division of *L. blattarum* is very similar to that of *Polymastix melolonthæ*, while the superficial periplast may show longitudinal markings.

Genus: Hexamastix Alexeieff, 1912.

This genus was created by Alexeieff (1912*b*) for a flagellate of the intestine of the newt, *Triton tæniatus*. The flagellate resembles in all essential respects a member of the genus *Eutrichomastix*, except that there are six flagella. It was first placed by Alexeieff (1911) in the genus *Polymastix*, from which he removed it in 1912. It may be related to the forms of *Trichomonas* with five anterior flagella.

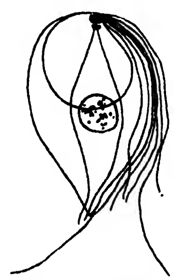


FIG. 284.—*Cochlosoma anatis* KOTLAN, 1923, FROM THE CÆCUM OF THE DUCK (× 2,000). (AFTER KOTLAN, 1923.)

Genus: Cochlosoma Kotlan, 1923.

This genus was created by Kotlan (1923) for an ovoid flagellate with six flagella arising from a blepharoplast at the anterior end of the body (Fig. 284). Behind the blepharoplast was a single nucleus, while two fibres arising from the blepharoplast passed backwards through the cytoplasm, one on each side of the nucleus, to the posterior end of the body, through which they protruded. The characteristic feature of the flagellate,

however, was the presence on one face of the anterior region of the body of a circular depressed area, which resembled in some respects the sucking disc of species of *Giardia*. There is a single species, *Cochlosoma anatis*, which occurs in the intestine of ducks. The large forms measured 10 to 12 microns by 6 to 7 microns, while smaller forms were 5 to 9 microns by 3 to 6 microns. The flagella, which appeared to vary in number, but of which there were usually about six, were directed backwards over the body.

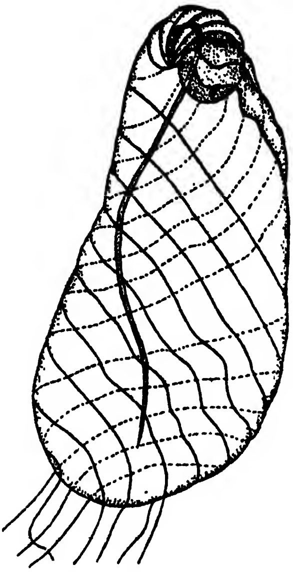


FIG. 285.—*Dinenympha gracilis* FROM THE INTESTINE OF *Termes lucifugus* (× 1,000). (AFTER ZULUETA, 1915.)

The flagellate possesses a single axostyle and nucleus, and a series of spirally arranged membranes with attached flagella.

10. *Family:* DINENYMPHIDÆ Grassi, 1911.

Amongst the numerous remarkable parasitic flagellates which occur in termites is a form which was placed in a separate family, the Dinenymphidæ, by Grassi to include *Dinenympha gracilis* Leidy, 1877 (Fig. 285). There is a single nucleus, a structure like an axostyle, and several flagella. The last arise from the anterior end of the body, are all directed backwards, and are attached to ridges producing an appearance of a series of undulating membranes which take a spiral course over the body. This flagellate evidently has affinities with *Trichomonas*, and forms a connecting link with the Polymonadida. Koidzumi (1921), who has named a number of new species, believes that the structure resembling the axostyle is in reality an elongate blepharoplast for the numerous flagella, as he could detect no separate blepharoplasts in the forms he examined. Comes (1912) believes that *D. gracilis* reproduces by multiple segmentation.

2. *Order:* HYPERMASTIGIDA.

This order (=Hypermastigina Grassi, 1911) includes a number of very complicated flagellates which are parasitic chiefly in the intestine of white ants (termites). There is a single nucleus and numerous flagella which arise from as many blepharoplasts. Axostyles and parabasal bodies may be present. *Lophomonas blattarum* Stein, 1860, occurs in the intestine of the cockroach. It is pear-shaped and possesses a single nucleus, in front of which are two groups of blepharoplasts, from each of which axonemes, giving rise to a tuft of flagella, orginate. An axostyle passes backwards from the

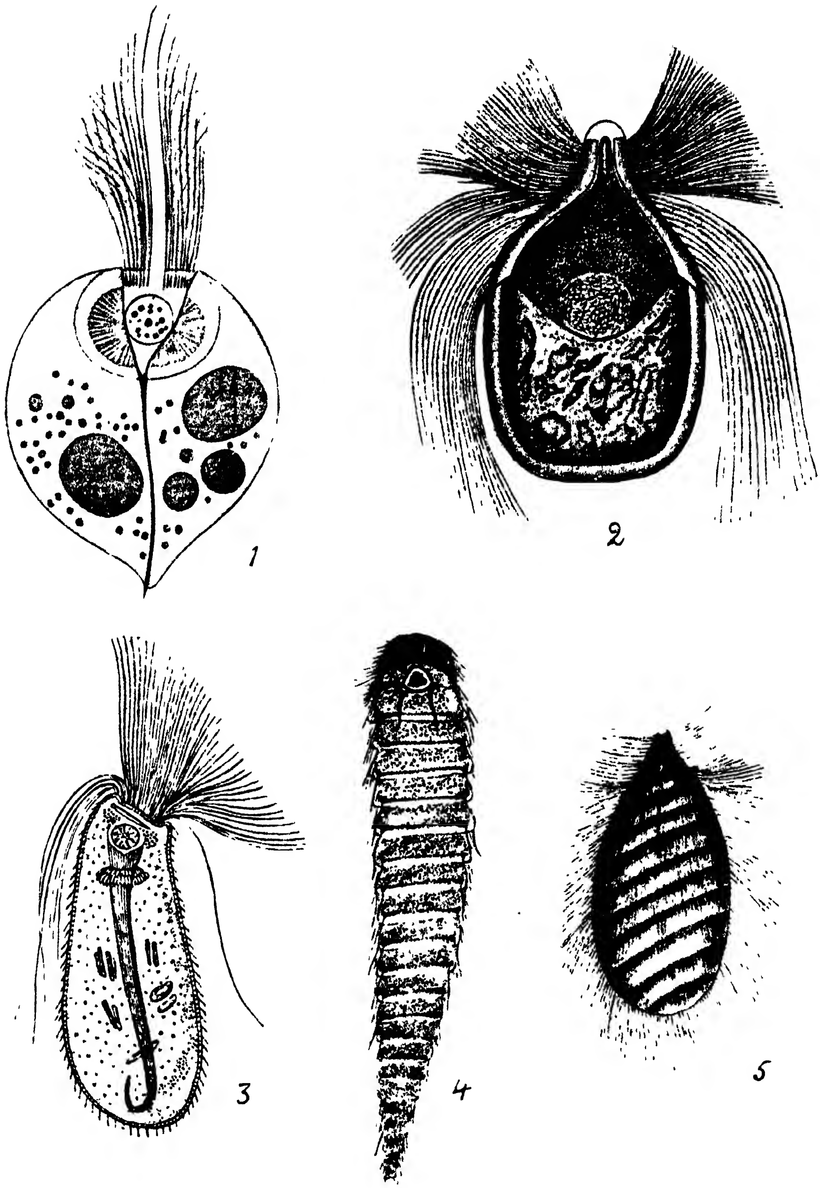


FIG. 286.—VARIOUS HYPERMASTIGIDA. (1, AFTER JANICKI, 1915; 2 AND 5, AFTER GRASSI AND SANDIAS, 1893; 3, AFTER BÜTSCHLI, 1889; 4, AFTER KOIDZUMI, 1921.)

1. *Lophomonas blattarum* of the cockroach (×2,900).
2. *Trichonympha agilis* of termites (× *ca.* 300).
3. *Jœnia annectens* of termites (× *ca.* 300).
4. *Teratonympha mirabilis* of termites (× *ca.* 300).
5. *Spirotrichonympha flagellata* of termites (× *ca.* 300).

blepharoplasts to the posterior end of the body, including in its course the nucleus, associated with which is a parabasal body. The Hypermastigida are subdivided into a number of families and genera, including the Trichonymphidæ, Leidy, 1877, which have since been studied by Grassi (1917), Kofoid and Swezy (1919), Koidzumi (1921), and others (Fig. 286).

3. *Order:* CYSTOFLAGELLATA Haeckel, 1873.

This order includes certain marine Protozoa, of which *Noctiluca miliaris*, a phosphorescence-producing organism, is the best known. The body is spherical, and may reach a diameter of over 1,000 microns. It has a groove leading to the cytostome, in front of which is a thick tentacle, with a length equal to half the diameter of the body, and a single flagellum. Reproduction is by binary fission or bud formation.

B. Diplozoic Forms.

4. *Order:* DIPLOMONADIDA.

The flagellates belonging to this order (=Diplozoa Hartmann and Chagas, 1910) differ from all others in that the nucleus and other organs are duplicated, so that the body has a bilateral symmetry. They may be supposed to have originated from certain uninucleate Protomonadida, which have commenced a division process that has been arrested before division of the body has taken place. The order contains the three genera: *Hexamita*, *Giardia*, and *Trepomonas*.

Genus: **Hexamita** Dujardin, 1841.

The flagellates of this genus have pear-shaped bodies provided with six anteriorly directed flagella, and two which arise from the posterior end. There are two nuclei at the anterior end of the body. The genus was founded by Dujardin (1841) to include three species, two of which occurred in stagnant water and one in the intestine and pectoral cavity of frogs and newts. He described the organisms as having pear-shaped bodies with four anterior and two posterior flagella, hence the name *Hexamita*. It appears that *H. inflata* of stagnant water is the type species of this genus, though Dujardin placed in the same genus, *H. intestinalis*, the parasitic form (Fig. 287). It is now known that the latter, as pointed out by Grassi (1879) for the form in the frog, in addition to the two posterior flagella, has six anterior ones, so that Dujardin evidently overlooked two of the latter. Dobell (1909) points out that there is little doubt that Dujardin was observing the eight-flagellate parasite, only six of the flagella of which he was able to count. If he made this error over the intestinal form, it is evident he was equally liable to make the same mistake as regards the type species, *H. inflata*, of stagnant water, for he places them in the

same genus. It is now known that the forms in stagnant water likewise have two posterior flagella, as well as six anterior ones, so that it seems evident the name *Hexamita* must be employed for these flagellates. Dobell (1909), though admitting that Dujardin overlooked two flagella in the intestinal form, apparently thinks he may not have done so in the case of the type species, *H. inflata*, though both were described at the same time and were regarded as having the same number of flagella. Dobell therefore adopts for the parasite of frogs the name *Octomitus*, proposed by Prowazek

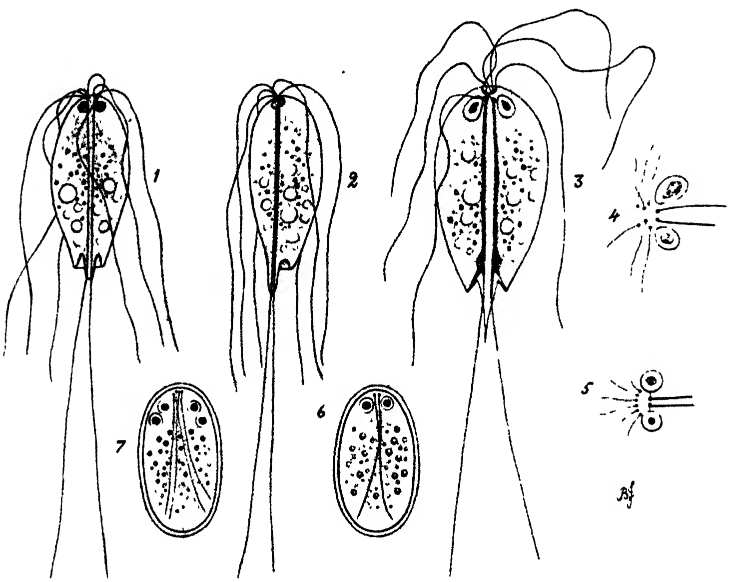


FIG. 287.—*Hexamita intestinalis* FROM THE RECTUM OF THE FROG (× 5,000). (ORIGINAL.)

1. Ventral view of living flagellate.
2. Side view of living flagellate.
3. Appearance in stained film.
4-5. Arrangement of nuclei and blepharoplasts as seen in flagellates stained by Giemsa stain after exposure to osmic acid vapour and drying.
6. Binucleated cyst.
7. Cyst after division of two nuclei.

(1904*a*). It seems to the writer that if it be accepted that Dujardin overlooked flagella in the intestinal form, as he undoubtedly did, it must be assumed he did so in the free-living form also. Klebs (1892), who first realized that the intestinal form had eight flagella, described as *Urophagus rostratus* a free-living form of similar structure, but which was said to possess a cytostome at the posterior end of the body. As pointed out by Alexeieff (1910), it seems very doubtful if he was correct in supposing a

cytostome to exist in this remarkable position. It seems more probable that he was observing species of *Hexamita,* in which had occurred some deformity of this part of the body, which is known to be very metabolic. Moroff (1903) proposed to employ Kleb's name, *Urophagus*, for these flagellates, owing to the uncertainty as regards the flagellates which Dujardin named *Hexamita.* There seems to be no doubt, however, that Dujardin was actually dealing with forms which are known to possess

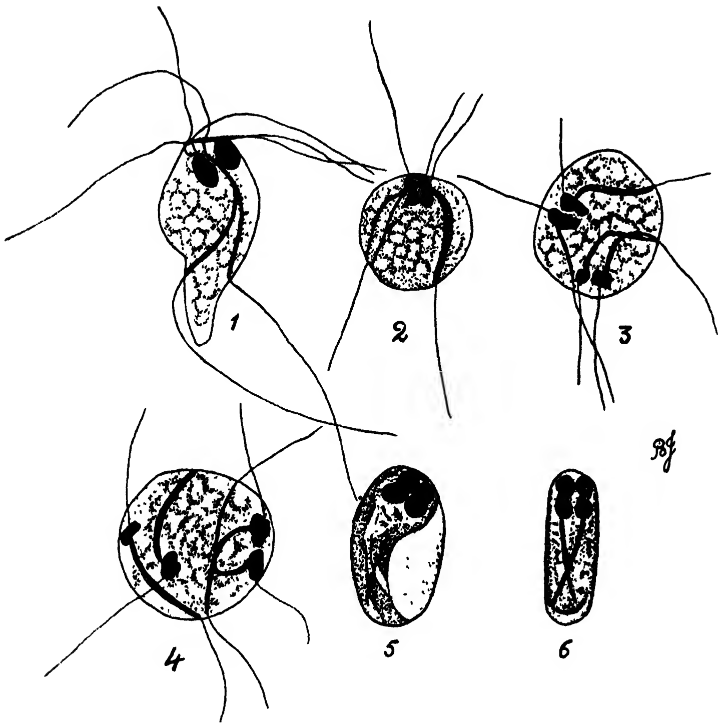


FIG. 288.—*Hexamitus muris* FROM THE INTESTINE OF THE MOUSE (×*ca.* 3,000). (AFTER WENYON, 1907.)

1. Ordinary free form. 2-4. Dividing forms.
5-6. Encysted forms, showing division of nuclei.

eight flagella, so that there is no reason why his name *Hexamita* should not be employed.

Hexamita muris (Grassi, 1881).—This species was first seen by Grassi as a parasite of the intestine of mice and other small rodents. It was named by him *Dicercomonas muris.* What is probably the same form was seen by Prowazek (1904*a*) in rats, and named *Octomitus intestinalis.* Lavier (1921*b*) records the flagellate from the field vole, *Microtus arvalis.* The organism was studied by the writer (1907). It has a rounded anterior

and a pointed posterior end, the latter being subject to changes in shape. In the small intestine of mice, the forms seen are 4 to 7 microns in length by 2 to 3 microns in breath. In the cæcum, longer and broader forms occur, which may measure as much as 10 microns by 5 or 6 microns. The latter may be adult forms of those found higher up in the intestine. From the anterior end arise six flagella in two groups of three (Fig. 288, 1). From the posterior end arise two flagella. In stained films it will be seen that the axonemes of the anterior flagella arise from two closely applied granules, each of which appears to be a compound structure composed of four blepharoplasts. From each granule there passes backwards a band-like structure, the axoneme, which is continued into a posterior flagellum. The axonemes of *Hexamita* are often referred to as axostyles, but there seems no reason to suppose that they are homologous with the axostyle of *Trichomonas*. In *Hexamita*, the axonemes usually stain deeply, while in *Trichomonas* the axostyle does not readily stain. It has been suggested by Kofoid and Swezy (1915*a*) that the axostyle of *Trichomonas* represents the axoneme of a backwardly directed flagellum, as in *Hexamita*. At the anterior end of the body of *H. muris*, and just behind the blepharoplasts, are two nuclei, between which the axonemes pass. Very frequently the nuclei, blepharoplasts, and anterior parts of the axoneme stain as a single compact and lobed mass, so that there is difficulty in distinguishing the separate parts (*cf*. Fig. 287).

Multiplication of *H. muris* takes place by longitudinal division (Fig. 288, 2-4). There is division of the blepharoplasts and nuclei, and with it division of the axonemes, so that there are produced rounded bodies with four nuclei and four axonemes. Presumably, by division of the body into two parts, two daughter individuals, each with two nuclei and two axonemes, are formed. Dobell (1909) has expressed it as his opinion that the division stages of *H. muris*, figured by Foa (1904) and by the writer (1907), were degenerate and fused forms which have nothing whatever to do with division. This is certainly not the case. Very similar division forms have been seen by Alexeieff (1908) and Swezy (1915) in species of *Hexamita* from amphibia (Fig. 290).

The encysted stages of *H. muris* also occur, and can be found in the cæcum. These are elongate bodies with rounded ends (Fig. 288, 5-6). They measure 6 to 7 microns in length by 3 to 4 microns in breadth. In stained films the cyst can be seen to contain a single flagellate. In some cysts, nuclear division has taken place, so that four nuclei are present.

If fæces of mice which are known to contain *H. muris* are diluted with water, cultures of this flagellate may be obtained. This seems to suggest that the forms which are found in stagnant water may actually be the same species as those which live in the intestine of amphibia and rodents.

Supposed Hexamita of Man.

Chalmers and Pekkola (1916) have recorded as *Octomitus hominis* a flagellate found by them in the human intestine in the Sudan (Fig. 289, 6.)

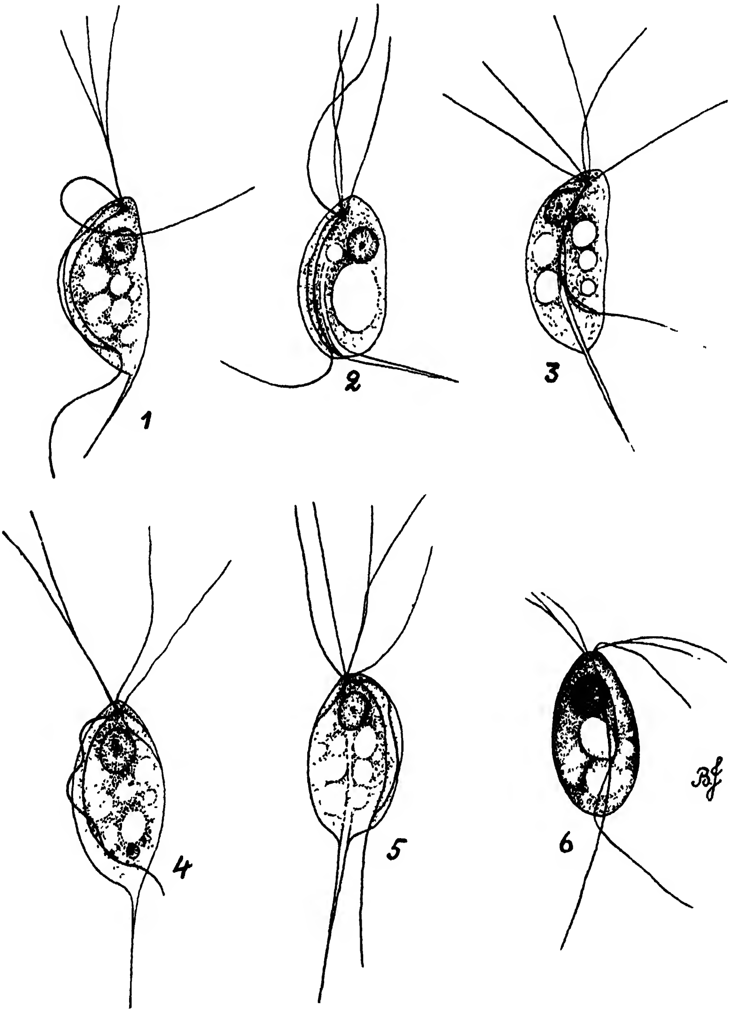


FIG. 289.—*Trichomonas* WITH FOUR AND FIVE FLAGELLA FROM A FILM SUPPOSED TO SHOW *Hexamita hominis* (× 3,600). (1-5, ORIGINAL; 6, AFTER CHALMERS AND PEKKOLA, 1916.)

1-5. *Trichomonas* with long drawn-out axostyle.
6. Chalmers and Pekkola's drawing of *Hexamita hominis*.

As this form possesses a single nucleus, and does not have the structure of *Octomitus* (*Hexamita*), doubts as to its validity were raised by Kofoid and

Swezy (1921*b*), who proposed establishing for it a new genus, *Ditrichomastix*, and by Dobell and O'Connor (1921), who suggested that it was possibly a dividing form of *Tricercomonas intestinalis*. From an examination of the original film, the writer is able to state that the supposed *Hexamita* is a *Trichomonas*. As is usual in a film, it is not possible to detect the complete structure in every flagellate, but there is no doubt that the infection is one of *Trichomonas*, and no other flagellate (Fig. 289, 1-5). The majority of forms in which the anterior flagella can be counted have four, a few have five, while others have a smaller number. The protruding portion of the axostyle in many is very long, while the basal fibre in some appears to be continuous with the posterior flagellum. In no case were six anterior flagella present, and it seems probable that some at least of the anterior flagella depicted by Chalmers and Pekkola were merely fibres in the medium.

Other Species of Hexamita.

According to Dobell (1909), the first observer to see a flagellate belonging to this genus was Ehrenberg (1838), who named a form seen by him in frogs, *Bodo intestinalis*. Dujardin (1841) named it *Hexamita intestinalis* and described two other species which he saw in stagnant water, *H. nodulosa* and *H. inflata*. Bütschli (1878) united the two latter forms under the name *H. inflata*, and called the parasitic one *H. intestinalis*. Grassi (1879) referred to the form in the frog as *Monomorphus ranarum*. The form described by Prowazek (1904*a*) as *Octomitus intestinalis* from the intestine of rats is certainly identical with *H. muris* of the mouse, while *O. dujardini*, described by Dobell (1909), from frogs and toads, is *H. intestinalis*. Moroff (1903) described a species of *Hexamita* from the rainbow trout. He regarded it as identical with the parasite of frogs and toads. Alexeieff (1910) observed a form in the fish, *Motella tricirrata* and *M. mustela*, and (1911) another in species of *Triton* and axolotl. These were all regarded as identical with *H. intestinalis* of the frog. *H. parva* is the name given to a form seen by Alexeieff (1912*c*) in the Ceylon tortoise, *Nicoria trijuga*. The writer has seen this or a similar form in *Testudo radiata*, *T. calcarata*, and *T. argentina*, and another in *Python molurus*. Mackinnon (1912) saw a form in the intestine of tipulid larvæ. Swezy (1915), who has given the most detailed account of the structure and division of these flagellates, described two new species (*H. ovata* and *H. batrachorum*), both from the intestine of amphibia (Fig. 290). Escomel (1925) gave the name *H. brumpti* to a form found in the South American batrachians, *Telmatobius escomeli* and *T. gebski*. Bělař (1916) described a species (*H. periplanetæ*) from the cockroach, Da Cunha and Muniz (1922), *H. avium* from Brazilian birds, and Kotlan (1923), *H. intestinalis* from the duck. Nöller and Buttgereit (1923) recorded *H. columbæ* from the

pigeon, and Da Cunha and Muniz (1925) *H. acuminata* and *H. elongata* from other birds of Brazil. Moore observed a flagellate in large numbers in the intestine of North American trout. She at first regarded it as a species of *Giardia*, and suggested the name *G. salmonis*. Later, both

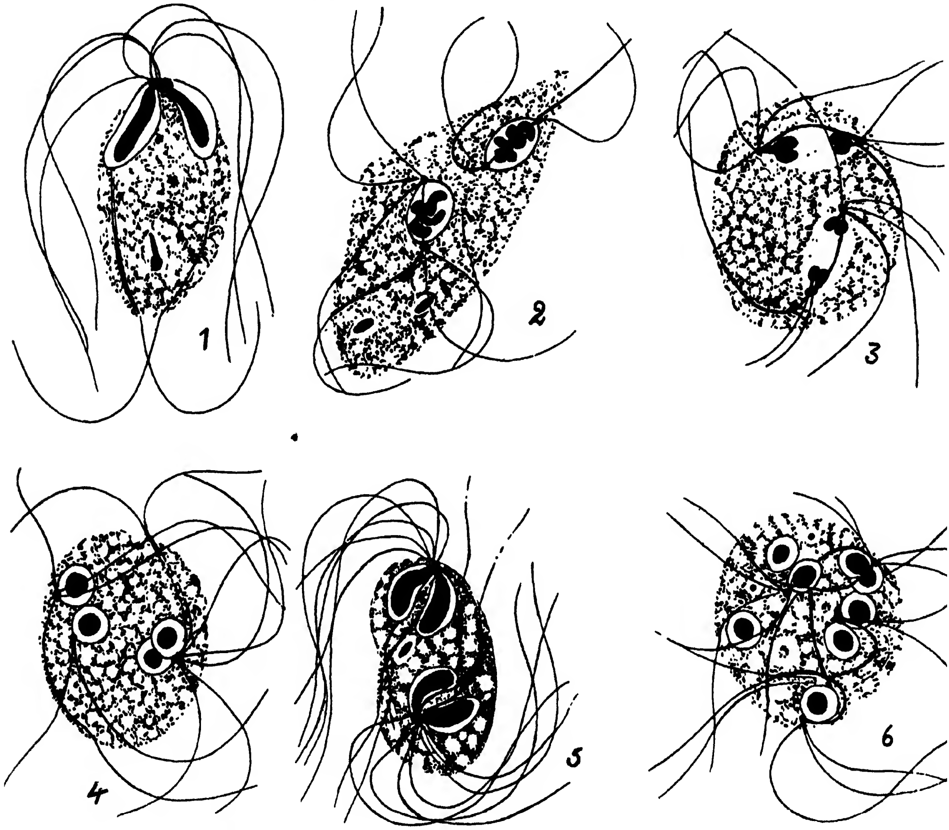


FIG. 290.—*Hexamita ovata* OF THE AMPHIBIAN, *Diemyctylus torosus*, SHOWING STRUCTURE AND METHOD OF DIVISION (× 2,583). (AFTER SWEZY, 1915.)

1. Normal flagellate, showing two nuclei, blepharoplasts, and axonemes of posterior flagella.
2. Early division stage: each nucleus has apical daughter blepharoplasts connected by a paradesmose; new axonemes and posterior flagella have developed; the chromatin is in the form of a spireme.
3. Later division stage: each nucleus has two groups of two daughter chromosomes; the paradesmose is still present.
4. Still later stage: the daughter nuclei are reconstructed, and the paradesmose has disappeared.
5. Stage just prior to division of the body.
6. Multiple division form.

Moore (1923) and Davis (1923) studied the flagellate in trout (*Salvelinus fontinalis* and *Salmo shasta*), and found that it in reality belonged to the genus *Hexamita*. They concluded that it invaded the intestinal cells, but the figures of the intracellular stages given by Davis are unconvincing.

Schmidt (1920) gave the name *Octomitus intestinalis truttæ* to a form from the intestine and gall bladder of European Salmonidæ.

Alexeieff (1917, 1917*a*) placed the forms seen by him (1910, 1912*c*) in the tortoise and fish in a new genus, *Octomastix*, as *O. parvus* and *O. motellæ*. Grassé (1924), who has seen the tortoise flagellate in the urinary bladder of *Emys orbicularis*, accepts this genus, the characters of which differ from those of *Hexamita* in minor details only.

Invasion of the Blood by Hexamita.

Danilewsky (1889) first pointed out that the *Hexamita* of frogs was able to invade the body cavity and even the blood-stream when the hosts were in poor condition. Plimmer (1914, 1916) observed flagellates of the *Hexamita* type in blood-films of tortoises (*Nicoria punctularia* and *Cistudo carolina*) which had died in the Zoological Gardens. Ponselle (1919) again observed a species of *Hexamita* in the blood of the edible frog, *Rana esculenta*. The infection was readily transmitted to other frogs (*R. temporaria*) by intraperitoneal inoculation of blood. Lavier and Galliard (1925) have also seen the parasite in the blood of frogs, but were unable to infect other frogs by inoculation.

Genus: Giardia Kunstler, 1882.

The members of this genus, which are all parasites of vertebrates with the single exception of a form discovered by Thomson, J. G. (1925), in a parasitic nematode (*Vianella* sp.), are characterized by the possession of two nuclei and a bilaterally symmetrical body, which is rounded anteriorly and tapered posteriorly. There is a dorsal convex surface and a flattened ventral surface, on which is a well-developed sucking disc with a raised edge circular in outline except at its posterior end, where it is indented to form a notch. There are eight flagella, four of which arise from the margin of the sucking disc, two from the posterior end of the body, and two from a median position in the notch of the sucking disc. The axonemes take a complicated course in the body.

A flagellate of this genus was first seen by Leeuwenhoek, who found himself infected in 1681, as pointed out by Dobell (1920). The human form was again seen by Lambl (1859), who called it *Cercomonas intestinalis*. Grassi (1879*a*) established the genus *Dicercomonas* with two sub-genera, *Monomorphus* (*Hexamita*) and *Dimorphus* (*Giardia*), but later (1881*a*) he replaced *Dimorphus* by *Megastoma*. He regarded the form in man as identical with others found by him in domestic animals. Blanchard (1888*a*) proposed the name *Lamblia*, which has been in general use for some years. Kunstler (1882), however, had established the genus *Giardia* for the flagellate seen by him in tadpoles, and there is little doubt that this is the correct generic name for these organisms.

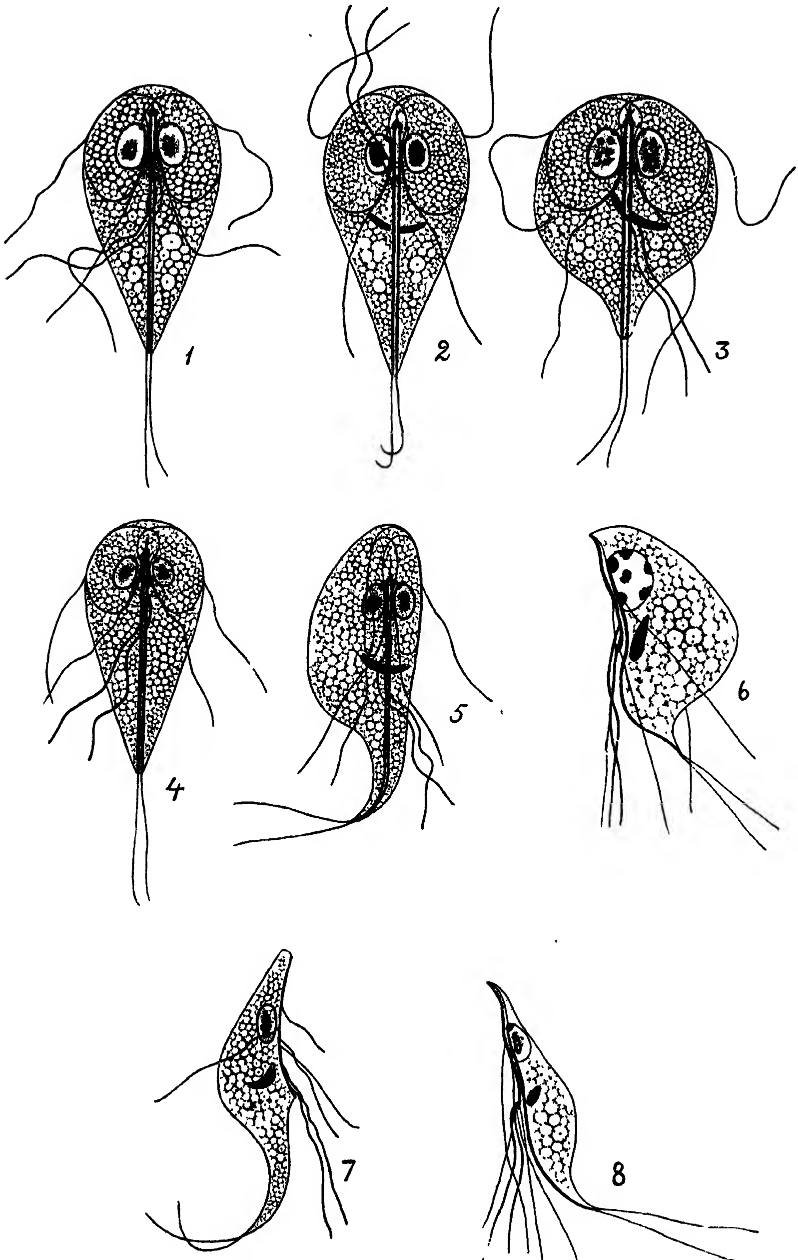


FIG. 291.—*Giardia intestinalis* FROM THE HUMAN INTESTINE (× 5,000). (ORIGINAL.)

1-4. Variations in size and shape of body. 5. Partial side view.
6-8. Variations in shape as seen in side view.

The members of the genus, which vary little in minute structure, are characterized by having a body which in shape resembles a longitudinally split pear. The dorsal surface is convex, while the ventral one is flat. The tapering posterior end or tail is a flexible structure which can be turned up over the convex dorsal surface. The rest of the body is rigid. On the ventral surface is the sucking disc, which is almost circular in outline save for a posterior indentation or notch. It has a raised edge, and by its means a flagellate is able to rest attached to the surface of epithelial cells. The four pairs of flagella, which are symmetrically arranged, originate in a series of blepharoplasts, the exact distribution of which has been variously described by different observers. Two nuclei are present, one lying on each side of the middle line of the body. There is no cytostome, though some observers incorrectly refer to the sucking disc by this name. Reproduction is by binary fission, which usually takes place within an ovoid cyst. Occasionally, division occurs in the unencysted condition. The body of a typical representative of the genus is distinctly flattened dorso-ventrally, though the degree of convexity varies considerably (Fig. 291). In some, which are probably the products of a recent division, the body is not more arched than a watch-glass, while in others, which are fully grown, it is almost hemispherical. When swimming in fluid media, the flagellate sways from side to side as any flattened object does when progressing through a liquid. The exact arrangement of the flagella, blepharoplasts, and axonemes is difficult to elucidate; so much so that the various observers who have undertaken the study of these flagellates have given different accounts. The difficulty of interpretation refers particularly to the region between the nuclei. The writer (1907) described what he considered to be the arrangement in *Giardia muris* of mice, and subsequent observations on *G. intestinalis* of man and other forms convince him that his original description was substantially correct. In the internuclear region the structures are so closely packed that the separate blepharoplasts cannot be recognized except in specimens which have been almost completely discoloured after staining with iron hæmatoxylin. Ordinary dried films stained by Giemsa stain not infrequently show the granules and axonemes distinctly, especially in individuals which have been flattened or even fortuitously dissected. Thin sections of the intestine in which the flagellates have been cut often show the structures more clearly than in flagellates mounted whole. Kofoid and Swezy (1922), Simon (1922), and Hegner (1922) state that there is only a single anterior blepharoplast on each side, but it appears from their figures that the single elongate blepharoplast is really composed of at least two closely applied blepharoplasts. They suppose that when two are present on each side this is an indication of commencing division. The following, in the

writer's opinion, appears to be the arrangement: All the blepharoplasts and axonemes have a superficial position on the ventral surface of the body. Between the two oval nuclei, which are also near the ventral surface, and slightly anterior to them, are four blepharoplasts arranged in pairs on each side of the middle line of the body. The lateral blepharoplast of each pair is slightly posterior to the one which is more centrally situated. Unless the stain is sufficiently extracted, each pair appears as a slightly elongated single blepharoplast. From the lateral ones there arise two axonemes, which pass forwards and, taking a curved course, cross one another. They reach the border of the sucking disc, pass along it for some distance, and finally enter flagella at points on its outer margin. From the median blepharoplasts there also arise two axonemes, the so-called axostyles, which pass backwards either on the surface of the body or just beneath it to the posterior extremity, to be continued into the posterior flagella. From these anterior central blepharoplasts there also arise two fibres which pass forwards and towards one another. They unite after a short course, and are continued as a single fibre, which is lost in the cytoplasm of the anterior part of the body. The single fibre sometimes appears as a group of radiating fibres. There is another pair of blepharoplasts centrally placed on the surface of the body in the hollow of the notch in the sucking disc. From them arise two axonemes which immediately enter flagella, which appear to arise directly from the blepharoplasts.

It seems probable that there is still another pair of blepharoplasts, from which the axonemes of the fourth pair of flagella originate. The axonemes of these can be traced forwards along the margins of the notch in the sucking disc, and can often be seen to terminate in a pair of granules at the anterior end of the notch. These are not improbably the blepharoplasts, which, however, appear to be connected with the anterior lateral blepharoplasts by fine fibres. If the granules are not the blepharoplasts, then it must be assumed that the fibres which connect them with the anterior lateral blepharoplasts are continuations of the axonemes, and that they terminate in the blepharoplasts from which originate the axonemes of the anterior crossed flagella. Not infrequently, granules may be seen in stained specimens at the point of entry of the axonemes into the flagella. This is particularly true of the posterior flagella, but these granules probably indicate a thickening of the superficial layer of cytoplasm or periplast, and cannot be regarded as blepharoplasts. In the arrangement, as just described, there can be distinguished a pair of lateral crossed flagella, the axonemes of which arise from the anterior lateral blepharoplasts; a pair of lateral uncrossed flagella with axonemes arising from the same blepharoplasts, or more probably from others posterior to

them; a pair of posterior flagella, the axonemes of which originate in the anterior median blepharoplasts; and a pair of central flagella having axonemes arising from the central blepharoplasts. The last pair of flagella are thicker than the others, and usually lie parallel to one another on the surface of the body. As in the case of the axonemes of *Hexamita*, those of the posterior flagella of *Giardia* are often regarded as axostyles, but they cannot be homologized with the true axostyle of a *Trichomonas*. They undoubtedly represent the intracytoplasmic portions of the axial filaments of the flagella, and are thus true axonemes. Simon (1922) figures them as broad anteriorly at their attachment to the blepharoplasts and tapering to a point posteriorly. Actually, they are of uniform thickness throughout. Very frequently there occur two deeply-staining curved or rounded bodies, which lie side by side just posterior to the sucking disc and dorsal to the axonemes of the posterior flagella. They are of largest size in the fully-grown individuals. These bodies have been homologized by Kofoid and Christiansen (1915, 1915*a*, 1915*b*) with the parabasals of other flagellates, but there is little real evidence in support of this view. The position of the blepharoplasts, as described above, seems to the writer to be the true arrangement. Other observers have considered that the axonemes of the eight flagella are all traceable to an anterior group of four blepharoplasts, while Kofoid and Christiansen (1915) in the case of *G. muris* and Kofoid and Swezy (1922) in the case of *G. intestinalis* conclude that they all terminate in two, and suppose that the presence of four in this region indicates the first stage in a division process, each blepharoplast having divided to give rise to two. This view is supported by Simon (1922) and Hegner (1922, 1922*a*). From the appearances seen in *G. intestinalis* of man and *G. muris* of mice, as also other forms, the writer believes that the undividing flagellate actually has eight blepharoplasts, each of which gives origin to an axoneme of a flagellum, as described above (Fig. 290). On *a priori* grounds alone, it is highly probable that each flagellum has its own blepharoplast. This is true of flagellates generally, and the members of the genus *Giardia* are unlikely to be exceptions to the general rule. When the blepharoplasts lie close together they often stain as a single body, so that the individual blepharoplasts are difficult to detect.

Several observers have described two fibres connecting the anterior lateral blepharoplasts with granules on the anterior extremity of the nuclear membrane. Bensen (1908) figures them in *G. muris*, while Kofoid and Christiansen (1915, 1915*a*) describe in *G. muris* and *G. microti* a continuation of these fibres to the karyosomes of the nuclei. They are also figured by Kofoid and Swezy (1922) in *G. intestinalis*. The writer has seen in *G. intestinalis* and other forms actual fibres connecting the

anterior lateral blepharoplasts with the granules on the nuclear membranes, but he is not convinced that these fibres are continued to the karyosomes of the nuclei (Fig. 290).

The nuclei are two ovoid bodies which lie one on each side of the middle line of the body near its ventral surface in the region of the sucking disc. Each consists of a nuclear membrane, within which is a karyosome, usually elongate in form. In what appear to be older individuals, several separate chromatin masses united by a meshwork of fibres are present.

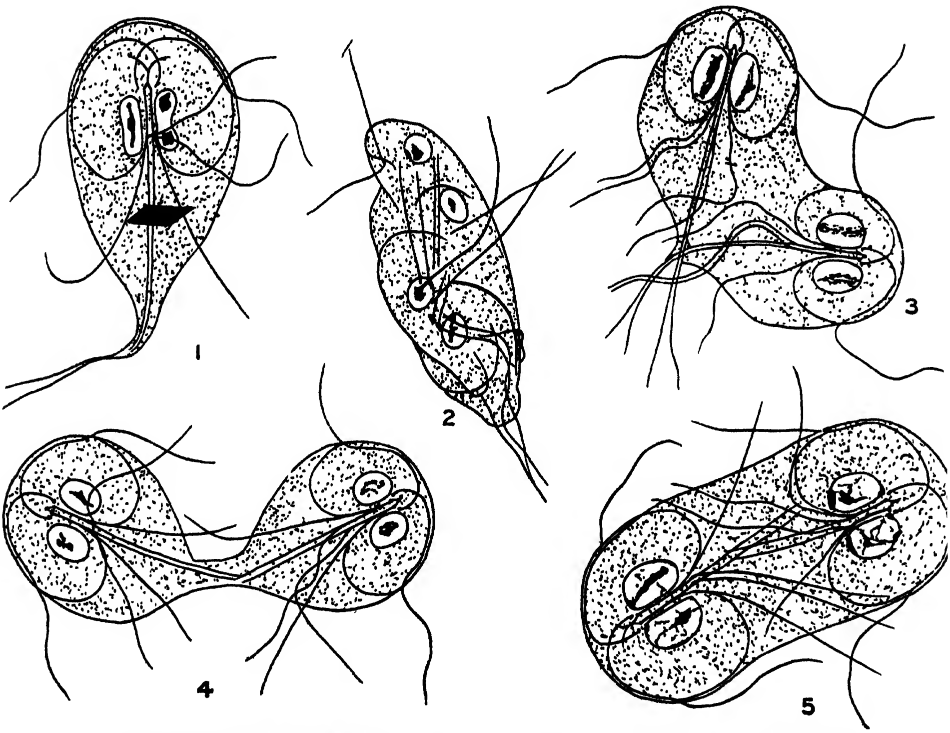


FIG. 292.—*Giardia intestinalis:* VARIOUS STAGES OF DIVISION (× *ca.* 6,000). (AFTER WENYON AND O'CONNOR, 1917.)

On the anterior surface of the membrane adjacent to the blepharoplasts is the granule mentioned above.

The flagellates multiply by a complicated process of longitudinal division. So seldom are division stages of the free flagellates encountered that most observers consider that the process occurs usually in the encysted condition. It seems probable that changes in the nuclei and blepharoplasts take place preparatory to division, which is completed within the cyst. The writer and O'Connor (1917) and Kofoid and Swezy (1922) obtained preparations of *G. intestinalis* of man which showed undoubted

division forms of the unencysted flagellates, while Kofoid and Christiansen (1915, 1915*a*) described a similar process of binary fission in *G. microti* and *G. muris*. It seems evident that binary fission may occur in the free condition, though most usually it takes place within the cysts, which are passed in large numbers in the fæces of infected individuals. The flagellates undoubtedly multiply in the intestine, and unless binary fission in the unencysted stage takes place more frequently than has been observed, it has to be assumed that the two flagellates which have resulted from division within the cyst are able to escape from the cyst without it leaving the host, a condition of affairs which is quite exceptional for intestinal Protozoa. In the case of most Protozoa, the encysted forms are destined to escape from the host in order to ensure infection of others.

The stages of division of *G. intestinalis* are shown at Fig. 292. It will be seen that the nuclei have divided, and that there has been duplication of the sucking disc and various blepharoplasts, axonemes, and flagella. The body finally divides from before backwards. The details of the process have not been worked out. It is evident that division within the cyst takes place in a similar manner, but here the various duplicated structures are so crowded together that it is impossible to follow the details with any degree of accuracy (Fig. 293).

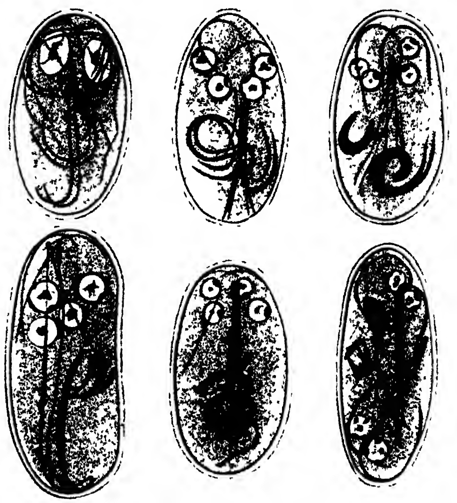


Fig. 293.—*Giardia intestinalis*: Encysted Forms from the Human Intestine (× 3,000). (Original.)

1. Form with two nuclei.
2-5. Forms with four terminal nuclei.
6. Form in which two of the nuclei have migrated to the opposite pole and the flagellate is dividing within the cyst.

The flagellate encysts in an ovoid cyst which forms first around the anterior end of the body. It extends backwards and gradually encloses the tail, which is finally retracted within the cyst. In recently encysted individuals the flagella and tail may be seen to be moving within the cyst. In stained specimens it will be noted that the two nuclei move to the anterior end of the body, where they divide to form four spherical nuclei. The fibre which forms the margin of the sucking disc becomes duplicated, and the two are often coiled in various ways. The blepharoplasts have each divided, and new axonemes and flagella have been developed, so that the cyst encloses an ovoid mass of cytoplasm containing four nuclei and

numerous fibres which are difficult to trace. One pair of nuclei moves to the opposite end of the cytoplasmic body, which divides longitudinally to form two flagellates. There is no evidence that two flagellates ever become encysted in a common cyst, as maintained by Schaudinn (1903), Bohne and Prowazek (1908), and Woodcock (1915). Hartmann's (1909) opinion that autogamy occurs in the cyst is likewise unsupported by fact.

In the smaller flagellates, which are probably the youngest forms seen in any infection, each nucleus has a single central karyosome (Fig. 294). In the larger or older individuals the karyosome is replaced by a number of granules distributed upon a meshwork. It is supposed by some observers that the formation of these granules is a preparation for nuclear division, and that ultimately eight chromosomes are formed. Rodenwaldt (1912) described the nuclear division of *G. intestinalis*. He noted that the nucleus of the free flagellate contained either a central karyosome or

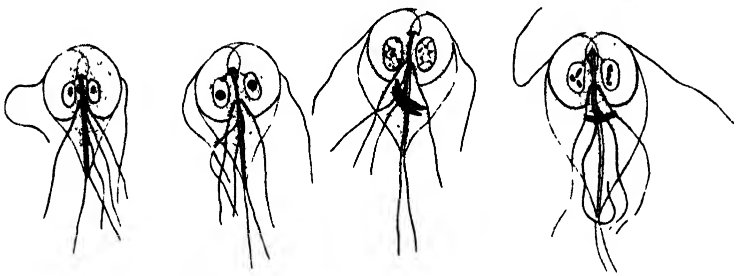


FIG. 294.—*Giardia intestinalis* OF MAN, TO ILLUSTRATE THE GROWTH OF THE FLAGELLATE, FROM A FILM IN WHICH DIVIDING FORMS WERE PRESENT (× *ca.* 1,500). (ORIGINAL.)

eight separate bodies. When a flagellate with a nucleus of the latter type encysted, the eight masses or chromosomes arranged themselves in two longitudinal rows, while the granules on the anterior end of the nuclear membrane divided into two. The nucleus then became constricted at its centre, and finally divided, each daughter nucleus receiving four of the chromosomes. If this be correct, it would appear that the division of the single karyosome in the flagellate stage into eight masses represents the commencement of division, which is completed after encystment has taken place. Kofoid and Christiansen (1915, 1915*a*) described binary fission of *G. muris* and *G. microti*, and Kofoid and Swezy (1922) that of *G. intestinalis*. The process bears a close resemblance to that seen in *G. intestinalis* by the writer and O'Connor (1917). The details of the nuclear division were studied by Kofoid and Christiansen (1915, 1915*a*), by Boeck (1917), and by Kofoid and Swezy (1922). The resting nucleus possesses a central karyosome. The first stage in division is supposed to be the division of the single pair of anterior blepharoplasts to produce

two pairs. The writer has already expressed it as his opinion that the flagellate possesses two pairs of anterior blepharoplasts, and he believes that when division is taking place there actually occur four pairs of anterior blepharoplasts. Boeck describes the changes in the nucleus as taking place in the following manner (Fig. 295): The fibril, which is said to connect the granule on the anterior end of the nuclear membrane with the karyosome, becomes extended to the opposite pole of the nucleus. The karyosome then becomes more irregular in shape, and finally divided into eight chromosomes. Meanwhile, the granule on the anterior end of

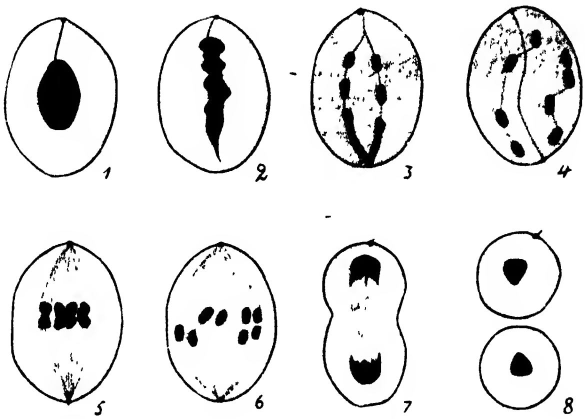


FIG. 295.—MITOTIC DIVISION OF NUCLEUS OF *Giardia microti* (× *ca.* 7,300). (AFTER BOECK, 1917.)

1. Ordinary resting nucleus with karyosome connected with centrosome by a fibril.
2. Karyosome has elongated.
3. Elongated karyosome has split longitudinally, and each half is dividing into four chromosomes.
4. Division of each half of karyosome into four chromosomes is complete. The centrosome has divided, and the two daughter centrosomes are connected by a fibre (paradesmose).
5. The eight chromosomes have united to form four double chromosomes at the equator of the spindle. The paradesmose is no longer visible.
6. The four double chromosomes have divided to form two groups of four which move towards the poles of the spindle.
7. Division nearly completed; chromosomes fused.
8. Completed division.

the nuclear membrane has divided, and one half migrates over the surface of the nuclear membrane to the opposite pole of the nucleus. It remains connected with the other half, which retains its anterior position, by a centrodesmose which lies on the surface of the nuclear membrane. Between the two granules, which are functioning as centrosomes, a spindle is formed within the nuclear membrane, and upon the fibres of the spindle the eight chromosomes arrange themselves. The chromosomes are described as four pairs of homologous chromosomes, and the individuals of each pair become closely associated to form four double chromosomes at the equator of the spindle. One chromosome of each pair now moves

towards the anterior centrosome, while the others pass to the posterior centrosome. The chromosomes of each group then fuse to form a karyosome, and this is followed by constriction and division of the nuclear membrane, so that two nuclei are formed. In the writer's experience, *G. intestinalis* of man frequently shows nuclear pictures, which correspond with all the stages figured by Boeck (1917) for *G. microti*, with the exception of the actual division of the nuclei. In such preparations, though very large numbers of flagellates are present, none of them is actually dividing in the free stage. If the various nuclear appearances undoubtedly represent a mitotic division of the nuclei, as first pointed out for *G. intestinalis* by Rodenwaldt (1912), one would expect to find more frequently a corresponding number of flagellates with two pairs of nuclei and others with their bodies actually in process of fission. It is possible, as Rodenwaldt maintains, that usually the nuclei of the free forms prepare for a division which is completed in the cyst. In one case noted above, the writer and O'Connor (1917) encountered numbers of free flagellates actually in process of binary fission (Fig. 292), and similar forms have been described by Kofoid and Swezy (1922), so that it has to be admitted that division in the free state can take place.

Kofoid and Christiansen (1915, 1915*a*) have described multinucleate stages of *G. microti* and *G. muris*. Both the free flagellates and the encysted forms are described as dividing by multiple segmentation. It is remarkable that in some of the figures the two normal nuclei of the free or encysted flagellates are in the position and possess the characters they usually have, while the other supposed nuclei are smaller and have a different appearance. It is difficult to understand how such a multinucleate condition can have arisen if the two normal nuclei are still in their usual situation, and are unaltered in size and appearance. The writer has seen very much vacuolated specimens of *G. intestinalis* having at the centre of each vacuole a granule which might be mistaken for a karyosome. It seems highly probable that it is structures such as these which have been interpreted as nuclei. Similar multinucleate cysts of *G. intestinalis* have been described by Kofoid and Swezy (1922). As many as sixteen nuclei are said to be present. In no case was division of the encysted form or daughter flagellates observed. Noc (1909*a*) gave an illustrated description of what he considered to be multiple division of *G. intestinalis*. It was supposed that after nuclear divisions a number of minute flagellates were produced, but it is evident from his figures that some of the forms depicted are not *G. intestinalis*, even if they are living organisms.

Various species of *Giardia* have been described from man and animals, but the specific characters are in most cases very ill-defined. Simon (1922)

and Hegner (1922*a*) maintain that species may be distinguished by the average dimensions of a number of individuals, and they illustrate in a graphic manner the measurements which are necessary for identification (Fig. 296).

Reuling and Rodenwaldt (1921) have attempted to revive the genus *Lamblia* by suggesting that *G. agilis*, described by Kunstler (1882) from tadpoles, differs sufficiently from the other forms to justify their inclusion in a separate genus, *Lamblia.* The tadpole parasite is a long narrow organism with a small sucking disc (Fig. 298), while all other forms rarely have a length as much as twice that of their breadth. They believe that the generic name *Giardia* should be retained for the narrow form, of which there is the one species, *G. agilis* Kunstler, 1882. The broader forms, which include all the others, are to be placed in Blanchard's genus, *Lamblia*, the type species being the human parasite, *L. intestinalis* (Lambl, 1859). The authors seem inclined to this view rather from a desire to retain the name *Lamblia* for the human parasite than from conviction that the differences between the two forms are of a generic value. It does not seem to the writer that matters are assisted in any way by splitting into two sub-genera the very compact and uniform genus, *Giardia*, merely because certain forms in the tadpole are narrower than those in other animals. As pointed out by Hegner (1922), the differences described are certainly not of generic value.

The various forms of *Giardia* which are known are invariably inhabitants of the small intestine. In mammals they are to be found in the upper parts of the small intestine and duodenum. When they occur lower down, it is probable that their appearance is accidental. They can be studied in sections of the intestine, and are often found in large numbers in the tubules of the secreting glands, a fact which probably affords an explanation of the difficulty in getting rid of an infection in human beings by the administration of intestinal disinfectants.

GIARDIA IN MAN.

Giardia intestinalis (Lambl, 1859).—As noted above, this flagellate was named *Cercomonas intestinalis* by Lambl (1859). Diesing (1851) had, however, given this name to a flagellate which Ehrenberg had previously described as *Bodo intestinalis*. As pointed out by Dobell (1909), Diesing was in error in so doing, as Ehrenberg's flagellate was not a *Cercomonas*, but probably a *Hexamita*. Hence, Dobell concludes that Lambl's specific name is still available for the human *Giardia.* Kofoid (1920), believing that Lambl's name was not available, adopted Grassi's name, and referred to the human form as *G. enterica* Grassi, 1881; while Simon (1922) states that Stiles has shown that the name *G. enterica*, which is in reality a synonym of *G. muris*, cannot be employed, and proposes to adopt the name *G. lamblia* Stiles, which was put forward in a paper by Kofoid and Christiansen (1915). If the rules of nomenclature are strictly adhered to, Lambl's specific name *intestinalis* cannot

be employed for the human *Giardia*, since the name *Cercomonas intestinalis* was already given to another flagellate (*Hexamita* of frogs, Diesing, 1851), when Lambl used it in 1859 for the *Giardia* of man. As Boeck and Stiles (1923) point out, it appears that the correct name for the human *Giardia* will have to be *Giardia lamblia* Stiles, 1915. The better-known name, *G. intestinalis*, will, however, be retained here.

This species is a common intestinal parasite of man, and has a world-wide distribution. It lives in the upper parts of the small intestine, thus differing from the other intestinal Protozoa of man, which are inhabitants of the large intestine, with the possible exception of the coccidia. Müller (1889) discovered it in the duodenum of one case at autopsy, an observation which was repeated by Moritz and Hölzl (1892). Cohnheim (1903, 1909) and Zabel (1901-1910) recorded what was probably *G. intestinalis* in stomach contents in cases of carcinoma. Boyd (1921) in Canada obtained large numbers of the flagellates by means of a duodenal tube passed on a convalescent typhoid case. A similar observation has been made by McGill (1922), Knighton (1922), Simon (1922), Silverman (1923), and Libert and Lavier (1923). As the bile obtained by the operation described as duodeno-biliary drainage contains large numbers of the organisms, it is concluded that they have actually invaded the bile ducts and gall bladder. This was confirmed by an observation of Smithies (quoted by Knighton), who found the flagellates in the gall bladder at surgical operation. Westphal and Georgi (1923) also record the discovery of *Giardia* in a gall bladder opened at operation. The writer has seen *G. intestinalis* in sections of the small intestine from fatal cases of typhus fever.

The general shape of the flagellate and arrangement of the various organs conform with the description given above (Figs. 291, 294). The length of the body, not including the tail flagella, varies from 10 to 18 microns, though longer and shorter forms sometimes occur. The breadth, which is a little more than half the length of the body, is subject to greater variations than the length. Simon (1922) gives the following measurements in microns for the flagellate: length 9·25 to 20·25 (average 13·7), breadth 5·0 to 10·25 (average 7·46).

Encysted forms are very commonly seen in the stools of infected individuals. It is only in diarrhœic conditions that the free forms are seen. The cysts are ovoid bodies varying in length from 8 to 14 microns (Fig. 293). Simon (1921) gives for the length 8·0 to 14·0 (average 10·7) microns, and for the breadth 6·0 to 10·0 (average 7·47) microns. In the fresh condition the cysts are quite transparent. With careful observation it is usually possible to distinguish the nuclei, the central axonemes, and some of the flagella. The nuclei are situated at the anterior end of the cyst, and each of these may have divided to form a total of four small spherical nuclei. In iodine solution or in stained films the

various structures are more readily detected (Plate II., 23, p. 250). Within the cyst the flagellate ultimately divides into two, but the process of division is an exceedingly complicated one on account of the numerous structures present. The cysts, like those of other intestinal Protozoa, vary in their permeability to stains and other reagents. On this account, good pictures of the cyst content are only obtained in the case of permeable cysts. As pointed out above, *G. intestinalis* is sometimes seen dividing in the free condition. As the flagellate possesses no cytostome and the cytoplasm is free from food vacuoles, it is evident that nourishment is effected by the absorption of fluid nutriment through the surface of its body. Bacteria are sometimes seen in evidently degenerate forms.

Pathogenicity.—The question of the pathogenicity of *G. intestinalis*, as that of other intestinal flagellates of man, has given rise to considerable controversy. It is an undoubted fact that the flagellates are rarely seen, except in diarrhœic conditions, but that they are often present in normal individuals can be demonstrated by the finding of cysts in the formed stool. The number of cysts present in the stools are subject to fluctuations. They may be absent from the stool for varying periods, and reappear again later. Certain individuals are known to have remained infected for many years without showing any symptoms, but this fact cannot be raised as an argument against the occasional pathogenicity of the flagellate, as the same condition frequently occurs in infections with *Entamœba histolytica*. In some cases of *Giardia* infection there occur periodic attacks of diarrhœa associated with the passage of large quantities of clear mucus, in which enormous numbers of free flagellates occur. It is difficult to avoid the impression that this mucus has been produced at that part of the intestine where the flagellates are most numerous, and is the result of irritation set up by their presence. It is possible that in certain individuals which are more susceptible than others, the attacks of diarrhœa correspond with periods of active multiplication of the flagellate.

In the case of animals, as, for instance, the rabbit, which is commonly infected with a species of *Giardia*, sections of the small intestine may show all the glands packed with organisms either free in the lumen of the duct or applied to the surface of the cells. When such a condition exists in man, it would not be surprising if the gland cells were irritated by the presence of such large numbers of flagellates. There does not appear to be any tendency for the flagellates to cause ulceration or to penetrate the epithelial surface. The majority of observers believe that *G. intestinalis* may give rise to intestinal disorders, but the absolute proof of this is difficult to obtain. The diarrhœic condition associated with an infection is often spoken of as dysentery, but actually true dysentery does not result. Though quantities of mucus may be present and the stools

diarrhœic in form, blood never occurs in pure *Giardia* infections. Westphal and Georgi (1923) have noted that in certain chronic disorders associated with jaundice the flagellates were present in large numbers in the duodenum, and in one case their presence in the gall bladder was demonstrated at operation. They believe that a definite inflammatory condition of the bile duct and gall bladder is set up by their presence.

Animal Experiments.—The fact that rats and mice are often infected with *Giardia* led Grassi (1879-1888) to express the opinion that human beings become infected from these animals. He claimed to have infected himself by means of the intestinal contents of rats. Perroncito (1901) stated that he had infected mice by feeding them with material from human cases, and Fantham and Porter (1916) made similar claims. The fact that mice are often naturally infected with *Giardia* renders such experiments very doubtful. Even prolonged examination of the fæces of the animals before the experiment may fail to exclude the natural infection. More recently, Deschiens (1921) has studied the question more fully. He was convinced that he had succeeded in infecting animals by means of human material. Thus, two cats were infected from human beings and two others from mice. All four animals developed a dysenteric condition which was fatal in three of the cases. Five mice naturally infected with *Giardia* failed to react to the human form, whereas five mice which were not naturally infected developed an infection with dysenteric symptoms, which proved fatal in three. The flagellates which appeared in the cats after ingestion of human material were said to be identical with those occurring after infection from mice. From these results Deschiens was inclined to regard *G. intestinalis* of man and *G. muris* of rats and mice as identical. Furthermore, he is convinced of the pathogenic rôle of these flagellates. It should be remembered, however, that both cats and mice are often found naturally infected.

Simon (1922) obtained white rats and wild rats which were free from *Giardia* infections. Attempts to infect them with *G. intestinalis* of man failed entirely, though they were readily infected with *Giardia* of mice. For this reason, and on morphological grounds, he concludes that the human infection is not contracted from rodents, but passes directly from man to man. The writer has attempted on several occasions to infect mice with the cysts of the human form, but has never succeeded. Quite recently he has conducted a carefully controlled experiment with four kittens. One of them had a natural *Giardia* infection. About 10 to 20 c.c. of fluid human stool containing numerous cysts was administered to each animal by means of an œsophageal tube. All four developed diarrhœa, and two actually passed blood and mucus. Cysts of *Giardia* were present in the fæces for two days, after which they disappeared, except in the case

of the naturally infected animal. Two of the cats died on the fourth day, when a careful examination of the intestines was made both in the fresh condition and in stained sections. There was no sign of any infection with *Giardia.* The naturally infected cat and one other survived for three weeks. They completely recovered from the intestinal disturbance caused by the inoculation. The naturally infected animal alone continued to pass cysts. The animals were killed, and, as was expected, *Giardia* was found only in the one which was already infected before the experiment.

GIARDIA IN ANIMALS.

As already remarked, a number of different species of *Giardia* have been described, but, with the exception of *G. agilis* of the tadpole, the various forms are very uniform in appearance. Simon (1922) and Hegner (1922*a*) have introduced a biometric method for the separation of species similar to that which has been employed in the case of trypanosomes. They maintain that if a sufficiently large number of individuals is measured, species can be separated by constructing curves showing the percentage of flagellates of any one size. Simon and Hegner claim that in this way it is possible to separate the human form from that of rats and mice, and Hegner those occurring in the dog and rabbit from each other and from those of human beings and rats (Fig. 296). Hitherto the occurrence of *Giardia* in different hosts has been the chief factor which has influenced observers in the establishment of species. Grassi (1879*a*) gave the name *Dimorphus muris* to the form in the mouse, while later (1881*a*) he noted the occurrence of *Giardia* in human beings and also in the cat, dog, rabbit, sheep, rat and mouse—*Mus musculus, Rattus rattus, R. decumanus, M. sylvestris, Arvicola* (*Microtus*) *arvalis*—and introduced the new name *Megastoma entericum.* Grassi and Schewiakoff (1888) added to the list of hosts *A.* (*Microtus*) *amphibius.* These observers regarded the animal forms as belonging to the same species as that occurring in man. In the writer's experience, *Giardia* is commonly present in cats, dogs, rats, mice, and rabbits in England. Kofoid and Christiansen (1915) have described as *G. microti* a form which occurs in *Microtus californicus* of California. They believe it to be distinct from *G. muris*, which they found, not only in mice, but also in *Peromyscus maniculatus gambeli.* Davaine (1875) described as *Hexamita duodenalis* a flagellate from the duodenum of the rabbit. It is undoubtedly a species of *Giardia*, so that the correct name for the form in the rabbit is *G. duodenalis*, though Bensen (1908) proposed to name it *G. cuniculi* (Fig. 296, *b*). Fonseca (1916) observed it in both the rabbit and *Cœndu villosus* of South America. A form he saw in the monkey (*Cebus caraya*) he regarded as identical with the human *G. intestinalis.* Splendore (1920) gave the name

G. pitymisi to a form occurring in the field vole, *Pitymis savii*. Simon (1922) has noted in *Microtus pennsylvanicus acadicus* in Nova Scotia a *Giardia* which appears to be identical with the form described by Kofoid and Christiansen as *G. microti* (Fig. 296, *f*).

Hegner (1923*a*) has recorded as *G. caviæ* a form found by him in the guinea-pig in America (Fig. 296, *g*). It is a small form like *G. muris*, but

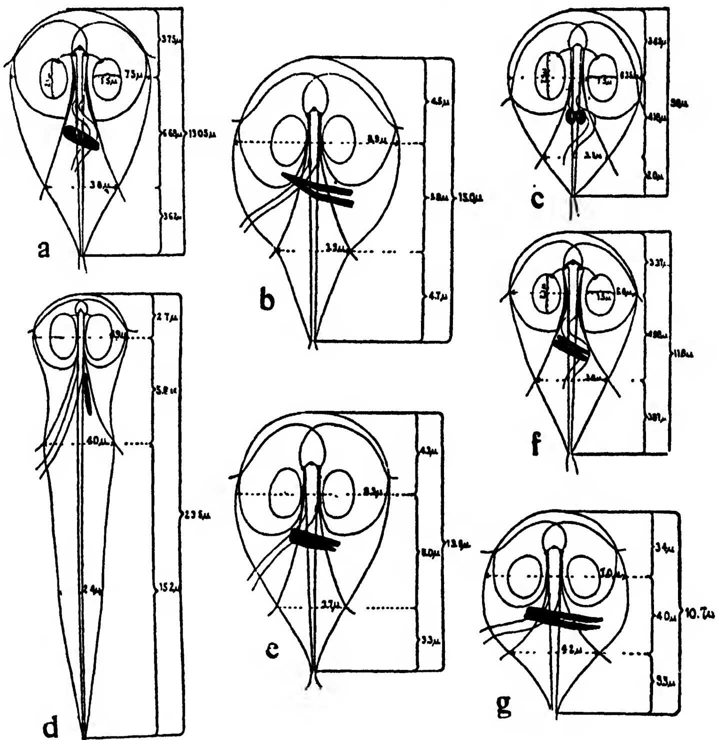


FIG. 296.—DIAGRAMMATIC REPRESENTATION OF VARIOUS SPECIES OF *Giardia*, SHOWING SPECIFIC DIFFERENCES. (FROM HEGNER AND TALIAFERRO, 1924, AFTER SIMON AND HEGNER.)

a, *G. intestinalis* of man; *b*, *G. duodenalis* of rabbit; *c*, *G. muris* of rats and mice; *d*. *G. agilis* of frog tadpoles; *e*, *G. canis* of the dog; *f*, *G. microti* of field mouse; *g*, *G. caviæ* of guinea-pig.

broader in proportion to its length, while the deeply staining bodies behind the sucking disc are represented by two rods which lie transversely and somewhat obliquely across the body. Another named species is *G. sanguinis* found by Gonder (1910*b*) in blood-films made from a falcon (*Elanus cœruleus*) which had been shot in the Transvaal. Nöller (1920*b*) described as *G. ardeæ* a form seen by him in the intestine of herons (*Ardea cinerea* and *Ardetta minuta*), while Kotlán (1922) discovered similar forms in the shrike (*Lanius collurio*) and avocet (*Recurvirostra*

avocetta). Later (1923), he recorded as *G. ardeæ* flagellates which he found in *Ardea cinerea*, *A. rubra*, *Nycticorax griseus*, and *Pelegadis falcinellus*.

Kunstler (1882), who founded the genus *Giardia*, described as *G. agilis* a form which occurs in the tadpole. Observing flagellates, which appeared to be of a different type in tadpoles, Kunstler and Gineste (1907) proposed to name two new species, *G. gracilis* and *G. alata*. Alexeieff (1914) expressed it as his opinion that all the forms belonged to the one species, *G. agilis*, a view which is shared by Hegner (1922), who has given a description of the tadpole flagellate.

As already remarked, apart from *G. agilis*, which is distinctly elongate, the various species of *Giardia* are very much alike in appearance, and the various morphological differences which have been described are quite

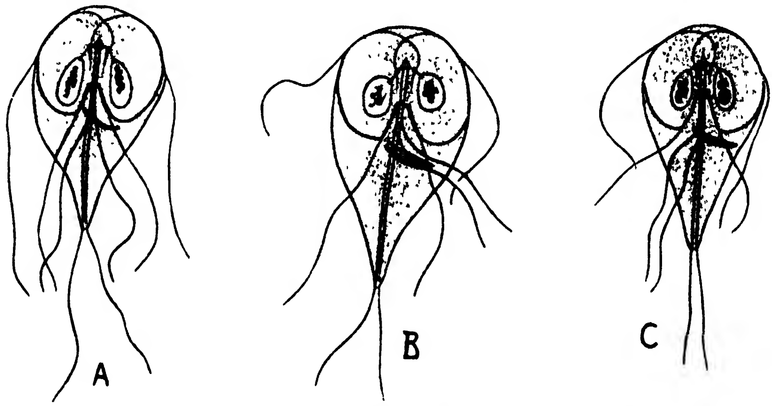


FIG. 297.—VARIOUS SPECIES OF *Giardia* OF MAMMALS (×2,300). (ORIGINAL.)
A. *G. muris* of the mouse. B. *G. duodenalis* of the rabbit. C. *G.* sp. from the cat.

inconstant and cannot be employed for the separation of species. If, however, a large number of flagellates from a human case be examined and compared with those occurring in rats or mice, the impression is gained that the human form is longer in proportion to its breadth than the mouse form. By actual measurement this is shown to be the case, and it is not improbable that a comparison of the average dimensions of the forms from other animals may show that constant differences in size occur, as Simon and Hegner maintain. Such a method of identifying species is, however, a long and tedious process.

Giardia muris (Grassi, 1879).—This species was first seen by Grassi (1879*a*, 1881*a*), who regards it as identical with the human form (Figs. 296, *c*, and 297, A). It was described by the writer (1907), and by Bensen (1908) and Kofoid and Christiansen (1915*a*). Bensen believed that it could be distinguished from other forms by certain morphological characters, especially those of the two deeply-staining bodies which lie dorsal to the

axonemes of the tail flagella. As pointed out by Simon (1922), none of these characters is of sufficient constancy to be of any value for separating species. According to him, it is only by the average dimensions that species can be recognized. His measurements for *G. muris* are: length, 7·25 to 12·75 (average 9·75) microns; breadth, 5·25 to 9·75 (average 7·26) microns. The dimensions of the cysts are very similar to those of *G. intestinalis* of man. Simon believes that in one white rat examined by him there occurred two distinct species. One of these was evidently *G. muris*. The other is referred to as *G.* sp. It was larger than *G. muris*, and varied in length from 10·25 to 16·75 microns (average 13·25) and in breadth from 6·25 to 9·25 microns (average 7·49). Both white rats and wild rats known to be free from natural infections were readily infected with *G. muris* from mice by Simon. He was unable to infect these animals with *G. intestinalis* or *G. microti*. Fantham (1925) records *G. muris* from *Rattus coucha* and *Tatera lobengula*.

White mice and rats are commonly infected with *G. muris*, which quickly spreads when introduced to a batch of these animals. There seems little reason to regard it as in any way pathogenic, though Kofoid and Christiansen (1915) maintain that the intestines of infected animals are altered to a yellow colour, which is most evident at the site of heaviest infection.

Hegner (1923*a*) has found *G. muris* in wild rats and mice in America. From a study of *Rattus norvegicus* in Paris, Lavier (1924) concludes that these rodents harbour two species of *Giardia*. One of these is *G. muris*, while the other appears to be morphologically identical with *G. intestinalis* of man. As all attempts to infect rats with the human *Giardia* have failed, Lavier believes that the form in the rat is a distinct species, for which he proposes the name *Giardia simoni*. It is apparently the form referred to by Simon as *G.* sp.

Giardia microti Kofoid and Christiansen, 1915.—This is a form which was discovered by Kofoid and Christiansen (1915) in meadow mice (*Microtus californicus californicus*) in California. They supposed that it could be distinguished from *G. muris* on morphological grounds, but Simon (1922) has shown that this is not the case, and that the species can only be distinguished by its measurements (Fig. 296). He gives these as: length, 8·25 to 13·75 (average 11·11) microns; breadth, 5·25 to 10·25 (average 7·58) microns.

Simon was unable to infect rats or mice with *G. microti* obtained from *M. pennsylvanicus acadicus*. It is not improbable that the form seen by Grassi in *Arvicola* (*Microtus*) *arvalis* in Italy, and studied by Lavier (1921*b*) in France, and that described by Splendore (1920) as *G. pitymisi* of *Pitymys savii* of Italy are identical with *G. microti*.

G. viscaciæ Lavier, 1923.—This species was discovered by Lavier (1923) in the viscacha (*Viscacia viscacia*), a rodent of South America.

The dimensions of the fixed and stained forms were given as 13 to 18 microns by 6·5 to 12 microns. The living forms appeared somewhat longer, and varied in length from 17 to 20 microns and in breadth from 9 to 12 microns. The cysts were 11 to 13 microns in length by 7 to 7·5 microns in breadth. Thomson, J. G. (1925), has made the interesting observation that intestinal nematodes (*Vianella* sp.) from the same rodents harbour what appears to be the same organism. Two of these rodents died in the Zoological Gardens in London. On examination, hundreds of nematodes heavily parasitized with the flagellate were found, though neither flagellates nor cysts could be discovered in the intestinal contents. The rodents also harboured numerous nematodes of the genus *Trichostrongylus*, but in none of these was the flagellate found. It seems highly probable that the flagellate is *G. viscaciæ*, which, being ingested by the worms, had found a habitat suitable for its multiplication. It is of interest to note that Brumpt (1910*a*) has observed that certain Ascaridæ parasitic in the colon of horses appear to feed exclusively on the Infusoria —ciliates and flagellates—which live in this part of the intestine.

G. duodenalis (Davaine, 1875).—This flagellate was first described by Davaine as *Hexamita duodenalis*. Grassi (1881*a*) regarded the rabbit form as identical with that of man, a view which was held by Metzner (1901). Bensen (1908) applied to it the name *Lamblia cuniculi*. The correct name is undoubtedly *G. duodenalis*. The rabbit flagellate has been studied by Hegner (1922*a*), who gives the measurements as follows: length, 12·7 to 18·7 (average 15·8) microns; breadth, 7·7 to 11·0 (average 9·1) microns. It is thus both broader and longer than *G. intestinalis* (Figs. 296, 297, B). The two deeply-staining bodies at the base of the tail are described as being often bent and longer than in other species. Fonseca (1915) described a form which he regarded as this species in *Cœndu villosus*, as also in the rabbit of Brazil. Hegner (1922*a*) believes that possibly he was dealing with a distinct species.

G. canis Hegner, 1922.—This form, which was first noted by Grassi (1881*a*), was again mentioned by Grassi and Schewiakoff (1888) and by Janowski (1897). The writer has seen it in dogs in England. Hegner (1922*a*) states that it has a characteristically broad anterior end (Fig. 296, *e*). It varies in length from 11·9 to 17·0 microns (average 13·8) and in breadth from 7·6 to 10·2 microns (average 8·5).

Giardia cati Deschiens, 1925.—This form was first seen by Grassi (1881*a*), and was named by Deschiens (1925). Later, Hegner (1925*a*) gave the name *G. felis* to a parasite of the cat in America. Hegner's flagellate, which may not be the same as the one studied by Deschiens, measured from 10·5 to 17·5 microns in length and from 5·25 to 8·75 microns in breadth. The cysts measured 10·5 by 7·35 microns. In the writer's

experience English cats are commonly infected. Hegner (1924) has seen cysts in the fæces of a wild cat, *Lynx ruffus*. They measured 11·01 to 13·55 microns by 6·57 to 8·47 microns. Fantham (1923) gave the name *G. suricatæ* to a form in the meercat, *Suricata tetradactyla*. Deschiens (1925*a*) has seen cyst of a species of *Giardia* in the fæces of two lions.

G. bovis Fantham, 1921, and **G. equi** Fantham, 1921.—These forms were recorded without details by Fantham, from the ox and horse in South Africa. Later (1923) he states that *G. equi* measures 20 by 10 microns, and the cysts 12 to 15 by 9·2 microns, and (1925) that the cysts of *G. bovis* measure 11 to 11·5 by 7 microns. Nieschulz (1923) found cysts of *Giardia* measuring 10 by 5·2 microns in the fæces of a calf in Holland.

G. capræ Nieschulz, 1923.—This form was discovered by Nieschulz (1923*b*, 1924*e*) in the goat in Holland. The free forms measured 9 to 17 by 6 to 9 microns. Cysts measuring 12 to 15 by 7 to 9 microns were also seen.

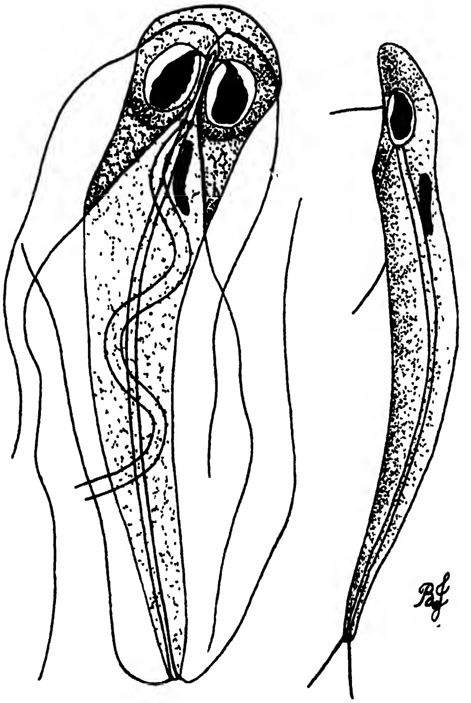


FIG. 298.—*Giardia agilis* OF THE TADPOLE (×4,300). (AFTER HEGNER, 1922.)

Hegner (1924) has seen the cysts of *Giardia* in the fæces of a monkey, *Atelus geoffroyi*. The measurements given are: length, 11·01 to 14·40 microns; breadth, 6·77 to 9·31 microns. It is stated that they are obviously different from the cysts of the human parasite. The writer has seen cysts of *Giardia* in a young monkey (*Cercopithecus*) from West Africa.

G. sanguinis (Gonder, 1910).—As pointed out above, this form was found by Gonder in the blood-films of a falcon shot in the Transvaal. In view of the fact that blood-films made from birds which have been shot frequently show contamination with intestinal organisms, there is little doubt that Gonder was dealing with an intestinal form which had contaminated the blood through the wounded intestine. Nöller (1920*b*) described as *G. ardeæ* an intestinal form from the herons, *Ardetta minuta* and *Ardea cinerea*. Kotlán (1922) has recorded *Giardia* from a shrike (*Lanius collurio*) and an avocet (*Recurvirostra avocetta*), while Rudovsky (1923) has found one in a buzzard.

Hegner (1925*a*) has seen giardias in the black-crowned night heron and the great blue heron in America, and Da Cunha and Muniz (1922) in *Ardea socoi*, *Cathartis aura*, and *Nycticorax nævius* in Brazil.

G. agilis Kunstler, 1882.—This form occurs in tadpoles, but the infection disappears when the metamorphosis into the frog takes place. The tadpole flagellate differs from all other known species of *Giardia* in the length of the body (Figs. 296, *d*, and 298). Hegner (1922) notes that structurally it differs in no respect from other species, though Reuling and Rodenwaldt (1921) described certain differences on account of which they suggested the retention of the name *Giardia* for this form and the name *Lamblia* for others. Hegner gives the measurements of *G. agilis* as follows: length, 14·4 to 28·9 (average 20·0) microns; breadth, 3·5 to 5·1 (average 4·5) microns.

Encysted forms have not been seen, though Alexeieff (1914) encountered small spherical cysts about 10 microns in diameter in a recently metamorphosed frog. He supposes the flagellates encyst soon after metamorphosis of the tadpole, and that the cysts, which he regards as those of *G. agilis*, escape into the water and are ingested by tadpoles in the following spring.

The form described by Fantham (1923) as *G. xenopi*, from the clawed frog, *Xenopis lævis*, may be the same species. He also records it from *Bufo regularis*.

G. denticis Fantham, 1919.—This flagellate was recorded by Fantham (1919) from the blood and intestine of the South African silver fish (*Dentex argyrozona*). It is not clear that the flagellates in the blood were not due to intestinal contamination. *G. salmonis*, recorded by Moore (1922) from trout in America, has been shown by Davis (1923) to be a *Hexamita* (p. 690).

G. varani Lavier, 1923.—This form was described and named by Lavier (1923) from the Nile monitor (*Varanus niloticus*). The length of the body varied from 15 to 21 microns and the breadth from 8 to 11 microns.

Genus: **Trepomonas** Dujardin, 1841.

This genus was established by Dujardin for a flagellate which occurred in sea-water infusions, and which he named *Trepomonas agilis*. Klebs (1892) also studied this organism and named other species. The writer and Broughton-Alcock (1924) have seen a form, probably *T. agilis*, on one occasion as a coprozoic flagellate in the stool of a human being suffering from mucous colitis (Fig. 299).

The organism is distinctly flattened and is oval in outline. There are two longitudinal grooves on the posterior half or two-thirds of the body, one on one surface and the other on the opposite surface. Sometimes they appear as if they are formed by a folding over of the edge of the body in

this region in such a way that one edge is folded forwards and the other backwards. An organism viewed from the anterior or posterior end has the appearance of an **S**, the hollows of the letter corresponding with the grooves. Running round the anterior end of the body is a horseshoe-shaped structure tapering at its extremities, which lie at the commence-

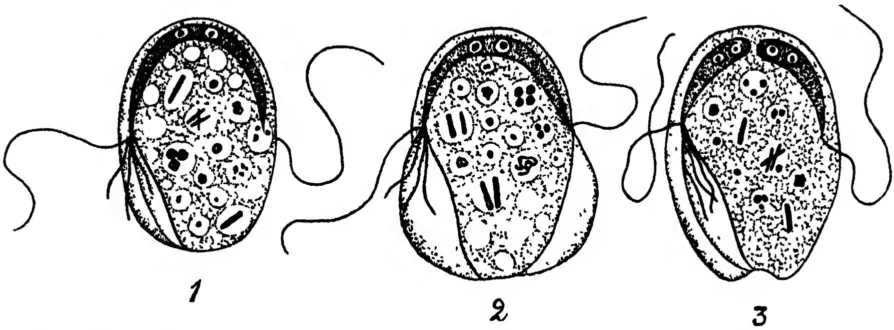


FIG. 299.—*Trepomonas agilis* AS A COPROZOIC FLAGELLATE IN HUMAN FÆCES (× 3,000). (AFTER WENYON AND BROUGHTON-ALCOCK, 1924; FROM *Trans. Roy. Soc. Trop. Med and Hyg.*, vol. xviii., p. 9).

1. Form with narrow groove.
2. Form with gaping groove with turned-out edges producing impression of lobes.
3. Early division form.

ment of the grooves. Within this structure can usually be distinguished four deeply-staining granules. Two of these lie near one another at the anterior end of the body, while the others are nearer its extremities. Arising from a point near the commencement of each groove are a number of flagella. One of these is a conspicuous long flagellum directed outwards, while the others are short and lie in the groove. There appear to be three short flagella, but it is not always possible to distinguish this number. Klebs figured a long flagellum and three short ones.

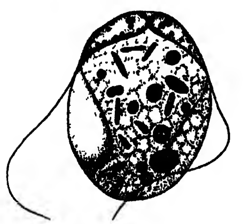


FIG. 300.—*Trepomonas* sp. FROM RECTUM OF MARINE FISH, *Box salpa* (× 2,250). (AFTER ALEXEIEFF, 1910.)

The nature of the horseshoe structure is doubtful. In dividing forms it splits into two, and one half moves to the opposite end of the body. Before it divides, however, division of the granules within it takes place, so that it is possible that the two anterior granules which are surrounded by a clear area are the true nuclei, the other two granules. blepharoplasts, and the structure itself a parabasal. The organism would appear to be related to *Hexamita*, the six short flagella in the grooves corresponding with the six anterior flagella and the two long ones with the posterior flagella.

The degree of development of the grooves varies considerably. Sometimes each is a narrow slit, while at other times it is wide and gaping. The margins, which are folded over, may be turned back, producing the appearance of

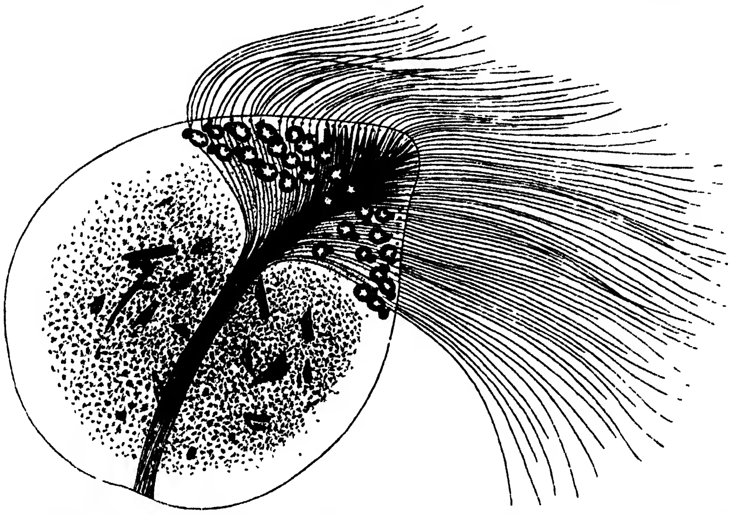


FIG. 301.—*Calonympha grassii* (× 1,600) FROM THE INTESTINE OF THE TERMITE, *Calotermes grassii*. (AFTER JANICKI, 1915.)

two large curved lobes one on each side of the posterior region of the body. The inner margin of each groove often appears to be strengthened by a fibre which passes round the posterior end of the body, where a notch sometimes occurs, and has its ends on the extremities of the horseshoe body. It sometimes appears as if this fibre is an actual continuation of the latter structure.

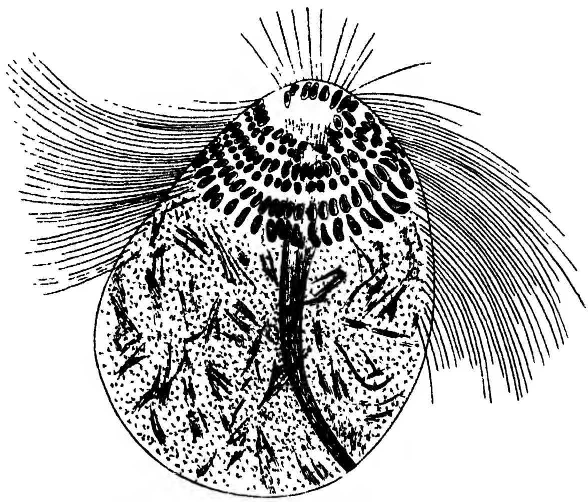


FIG. 302.—*Stephanonympha silvestrini* (× 1,200) FROM INTESTINE OF TERMITE, *Calotermes castaneus*. (AFTER JANICKI, 1915.)

Alexeieff (1910) discovered a form in the rectum of the marine fish, *Box salpa* (Fig. 300). The form he described was evidently a dividing flagellate, and only the two long flagella were depicted.

C. Polyzoic Forms.

5. *Order:* POLYMONADIDA.

The flagellates included in this order are polyzoic, and possess many nuclei and blepharoplasts, each of which gives origin to one or more flagella. In association with each nucleus, there may be a parabasal, while an axostyle is present. The members of this order may be supposed to have been derived from flagellates of the *Eutrichomastix* type, in which multiplication of nuclei and organs has taken place without division of the body. The order includes the single family *Calonymphidæ*, founded by Grassi for certain flagellates of termites which have the above characteristics. The family includes several genera, such as *Calonympha* (Fig. 301) and *Stephanonympha* (Fig. 302).

FREQUENCY OF INTESTINAL FLAGELLATE INFECTIONS OF MAN.

Human beings are commonly liable to infection with the following five intestinal flagellates: *Giardia intestinalis*, *Chilomastix mesnili*, *Trichomonas hominis*, *Embadomonas intestinalis*, *Tricercomonas intestinalis* (Fig. 303). The flagellated organism described by Kofoid and Swezy as a species of *Craigia*, but which is probably a species of *Sphæromonas* or *Oikomonas*, possibly identical with *S. communis*, described by Liebetanz (1910) from the rumen of cattle, is undoubtedly of rare occurrence. The last-named organism has been seen only by Kofoid and Swezy in five persons resident in America, and in one person who had returned from India (see p. 295). *Tricercomonas intestinalis* was seen in about a dozen cases of diarrhœa by the writer and O'Connor (1917) in Egypt. It is a small flagellate which is exceedingly difficult to identify. It was seen by the writer again in several cases of diarrhœa in Macedonia in 1918. Kofoid, Kornhauser, and Plate (1919) record three cases of infection in soldiers returned to America from abroad. The possibility of the identity of this flagellate with the form described as *Enteromonas hominis* has been discussed above (p. 653). It is probable that it is of fairly common occurrence, as recent observations have extended its known distribution. The difficulty of identifying it accurately may lead to its being regarded as a small form of *Trichomonas hominis*, *Chilomastix mesnili*, or even *Embadomonas intestinalis*. The last-named flagellate was seen by the writer and O'Connor (1917) in two cases in Egypt. It was again recorded by Kofoid, Kornhauser, and Plate (1919) in four patients returned to New York from overseas, and in four others who had never left the United States. A case was also seen by Hogue (1921*b*) in the same country, while another was seen by Broughton-Alcock and Thomson, J. G. (1922*a*), in a person who had returned to England from abroad. It has since been recorded from other localities.

The species of *Giardia*, *Chilomastix*, and *Trichomonas* are more extensively known, and can be considered to be world-wide in their distribution. As the two former can be recognized by their cysts, they can be detected in the formed as well as the diarrhœic stool, whereas the latter is only rarely seen in the formed stool owing to the absence of the encysted forms.

G. intestinalis was found by the writer and O'Connor (1917) to be present in 4·1 to 16 per cent. of normal individuals in Egypt. Dobell (1921) estimates that 18 to 27 per cent. of the artisan population of the British Isles harbour this flagellate, while Boeck (1921) found it present in 48·1 per cent. of eighty-three industrial school children examined in America.

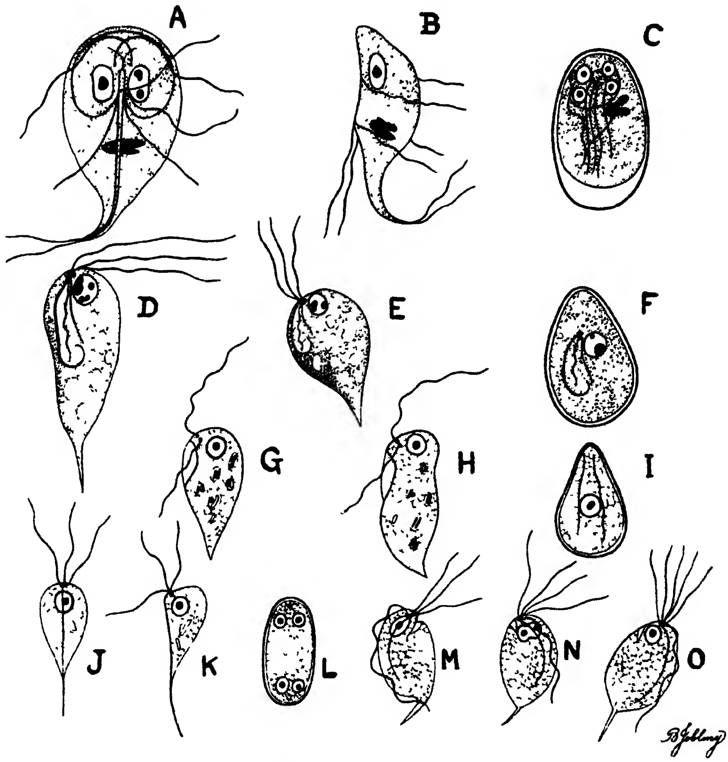


FIG. 303.—THE FLAGELLATES OF THE HUMAN INTESTINE (× 2,000). (AFTER WENYON, 1922.)

A-C. *Giardia intestinalis*, free and encysted forms.
D-F. *Chilomastix mesnili*, free and encysted forms.
G-I. *Embadomonas intestinalis*, free and encysted forms.
J-L. *Tricercomonas intestinalis*, free and encysted forms.
M-O. *Trichomonas hominis*, forms with three, four, and five flagella.

As regards *C. mesnili*, the writer and O'Connor (1917) obtained a percentage of 3·2 of infections amongst normal individuals in Egypt. For the population of the British Isles, Dobell gives 6 to 9 per cent., while Boeck gives 1·2 for American school children.

T. hominis was seen by the writer and O'Connor in only 3 per cent. of hospital cases in Egypt, and in a much smaller percentage of healthy people. Amongst the population of Britain, Dobell mentions that it was only occasionally seen, while it was not met with at all by Boeck in his examinations of American school children.

III. *CLASS:* CNIDOSPORIDIA DOFLEIN, 1901.

CLASSIFICATION.

CLASS: **CNIDOSPORIDIA**

- *Order:* MYXOSPORIDIIDA
  - *Sub-Order:* **Eurysporea**
    - *Family:* CERATOMYXIDÆ
      - *Genus:* **Leptotheca**
      - ,, **Ceratomyxa**
      - ,, **Myxoproteus**
      - ,, **Wardia**
      - ,, **Mitraspora**
  - *Sub-Order:* **Sphærosporea**
    - *Family:* CHLOROMYXIDÆ
      - *Genus:* **Chloromyxum**
      - ,, **Agarella**
    - *Family:* SPHÆROSPORIDÆ
      - *Genus:* **Sphærospora**
      - ,, **Sinuolinea**
  - *Sub-Order:* **Platysporea**
    - *Family:* MYXIDIIDÆ
      - *Genus:* **Myxidium**
      - ,, **Sphæromyxa**
      - ,, **Zschokkella**
    - *Family:* MYXOSOMATIDÆ
      - *Genus:* **Myxosoma**
      - ,, **Lentospora**
    - *Family:* MYXOBOLIDÆ
      - *Genus:* **Myxobolus**
      - ,, **Henneguya**
      - ,, **Hoferellus**
- *Order:* MICROSPORIDIIDA
  - *Sub-Order:* **Monocnidea**
    - *Family:* GLUGEIDÆ
      - *Genus:* **Glugea**
    - *Family:* NOSEMATIDÆ
      - *Genus:* **Nosema**
      - ,, **Perezia**
      - ,, **Gurleya**
      - ,, **Thelohania**
      - ,, **Stempellia**
      - ,, **Duboscquia**
      - ,, **Plistophora**
    - *Family:* COCCONEMIDÆ
      - *Genus:* **Cocconema**
    - *Family:* MRAZEKIIDÆ
      - *Genus:* **Mrazekia**
      - ,, **Octosporea**
      - ,, **Toxonema**
      - ,, **Spirillonema**
  - *Sub-Order:* **Dicnidea**
    - *Family:* TELOMYXIDÆ
      - *Genus:* **Telomyxa**
- *Order:* ACTINOMYXIDIIDA
  - *Genus:* **Tetractinomyxon**
  - ,, **Hexactinomyxon**
  - ,, **Triactinomyxon**
  - ,, **Synactinomyxon**
  - ,, **Sphæractinomyxon**

Parasites of Undetermined Position

SARCOSPORIDIA
GLOBIDIUM
HAPLOSPORIDIA

The Protozoa belonging to this class are amœboid organisms during the growing or trophic phase of development, while dissemination is effected by means of resistant spores, which are peculiar in being provided with one or more polar capsules. The latter, under certain conditions of stimulation, as, for instance, those of the intestinal fluids, extrude long filaments which are supposed to attach or anchor the spore to the intestinal wall till the enclosed amœboid body, the actual infective agent, is able to escape from the spore and invade the tissues of the new host. Gluge (1838) was the first observer to see small spores of one of these parasites in fish, but Johannes Müller (1841) discovered much larger ones in a number of fish, and referred to them as psorosperms, a name which was long used for them and the spores of coccidia and gregarines. The Cnidosporidia are often grouped with the Sporozoa, which Schaudinn (1900) divided into two sub-classes, the Telosporidia and the Neosporidia, the former to include the coccidia and gregarines, and the latter the Myxosporidiida, Microsporidiida, Actinomyxidiida, and Sarcosporidia The Telosporidia, however, have little in common with the Neosporidia. They have definite intracellular stages, reproduction is by schizogony, while the zygotes resulting from a conjugation of gametes become encysted in resistant oöcysts, within which they give rise to sporozoites. The Myxosporidiida, Microsporidiida, and Actinomyxidiida, on the other hand, though sometimes intracellular parasites, reproduce mostly by binary fission and not by schizogony, while the zygotes do not become encysted, nor do they give rise to sporozoites. Furthermore, the very characteristic spores possessing polar capsules are produced. Very little is known about the affinities of the Sarcosporidia, but it seems clear from their comparatively simple spores that they are in no way related to the Cnidosporidia, which produce the highly complex spores provided with polar capsules. In their development the spores of Cnidosporidia differ fundamentally from those of all other Protozoa, the resistant or encysted stages of which are produced by a cell secreting a capsule round itself. Subsequently the entire cell or the products of its division survive. In the case of the Cnidosporidian spore a single cell divides to form several cells, some of which give rise to the polar capsules, others to the spore membranes, while one or two alone survive. The production of the spore involves the sacrifice of several cells for protective purposes, while no such sacrifice is associated with spore formation in other Protozoa. This difference led Emery (1909) and Ikeda (1912) to suggest that the Cnidosporidia are in reality Metazoa. Attention has been again called to this point by Dunkerly (1925), who sees in this differentiation of cells a process by which Metazoa may have evolved from Protozoa. It seems, therefore, best to follow Hartmann (1907), and separate the Myxosporidiida, Micro-

sporidiida, and Actinomyxidiida from the Sporozoa, with which Schaudinn first grouped them, and to place them in a distinct class for which the name Cnidosporidia, suggested by Doflein (1901) for the order, can be employed. The Sarcosporidia do not appear to be related either to the Sporozoa or the Cnidosporidia, and will be considered with other forms with doubtful affinities, as was done by Labbé (1899).

A typical member of the class commences its existence as a small amœboid body which has escaped from the spore in the intestine of the host. It makes its way to the tissue or body space in which its subsequent development will occur. Here it may grow into a multinucleate plasmodium through repeated nuclear divisions not being followed by division of the cytoplasm, or it may multiply by binary fission or possibly by multiple segmentation or gemmation, so that a large number of uninucleate forms is produced. In either case spore formation eventually occurs. In the multinucleate plasmodial forms certain of the nuclei become separated with a portion of cytoplasm as small round cells which lie in vacuoles in the plasmodium. These uninucleate cells (pansporoblasts) in the vacuoles then become transformed into spores, while the plasmodium continues to increase in size. In the uninucleate forms the spores arise from one of the uninucleate parasites (pansporoblasts). The process of development of the spore from the uninucleate body with the production of the polar capsules is a very complicated one, and the type of spore produced in the different genera of Cnidosporidia varies considerably. The ultimate infective agent within the spore is an amœboid body which has one or two nuclei. It has often been referred to as a sporozoite, but there is no evidence that it is homologous with the typical sporozoites of Sporozoa. The Cnidosporidia include three orders: MYXOSPORIDIIDA, MICROSPORIDIIDA, and ACTINOMYXIDIIDA.

A. *Order:* MYXOSPORIDIIDA.

In these forms the trophic or growing phase is a multinucleate plasmodium, which resembles an amœba in that it is motile and forms pseudopodia (Fig. 304). They are typically parasites of cold-blooded vertebrates, a large number of species infecting fish, amongst which they give rise to severe and fatal epidemics. In some cases they live as harmless amœboid organisms in the body spaces, such as the gall bladder, urinary bladder, or tubules of the kidney, where they float about or are attached to the walls by pseudopodia. It is in these forms that the complicated process of spore formation has been chiefly studied. In other cases they are definite tissue parasites, which may give rise to nodules, sometimes of large size, on the skin and gills or in the muscles and other organs (Fig. 305). In the tissues the parasite may grow actually

within a cell, which becomes much distended, as in the case of muscle fibres (Fig. 306, D). Many forms, however, develop in the intercellular

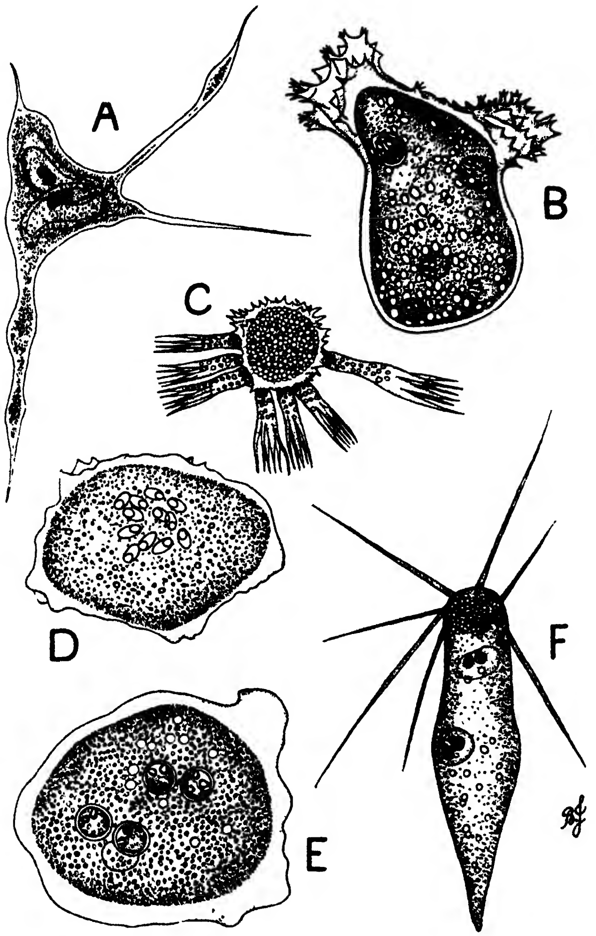


FIG. 304.—VARIOUS CNIDOSPORIDIA. (AFTER THÉLOHAN, 1894.)

A. *Ceratomyxa appendiculata* Thélohan, 1894, from gall bladder of *Lophius piscatorius* and *L. budegassa* Spores 50 × 5–7 μ.
B. *Chloromyxum leydigi* Ming., 1890, from gall bladder of various marine Elasmobranchs. Spores about 13 × 10 μ.
C. Several small forms of *Leptotheca agilis* Thélohan, 1894, attached to a specimen of *C. leydigi*.
D. *Glugea marionis* Thélohan, 1894, from the gall bladder of marine fish (Wrass), *Julis vulgaris* and *J. giofredi*. Spores 8 × 3 μ.
E. *Sphærospora divergens* Thélohan, 1894, from the kidney tubules of *Blennius photis* and *Crenilabrus melops*. Spores 10 μ in diameter.
F. *Leptotheca agilis* Thélohan, 1894, from the gall bladder of the ray, *Trigon vulgaris*. Spores 11–12 × 6-7 μ.

spaces, the tissues of the host and the parasites being closely intermingled (Fig. 306, A-C). The infected area of tissue is frequently shut off by the

development, on the part of the host, of a fibrous capsule, within which occur the remains of host cells with their hypertrophied nuclei, and the multinucleate plasmodia containing a varying number of spores. The central portion of such an encapsuled area, owing to degeneration of the central part of the parasite, may consist of granular débris and spores, while thec apsule itself is lined by the multinucleate cytoplasm of the parasite, which continues to grow and produce spores. In old nodules spores and débris alone may be detected, while later still fibrosis or even calcification may occur, and all trace of the parasites be lost.

Infection is brought about in the first place by the small amœboid organism, which frequently has two nuclei, escaping from the spore in the intestine of the host. It is claimed by some that at this stage syngamy takes place, but the evidence of this is conflicting. The amœboid organism, which is now known as a planont, makes its way to that par-

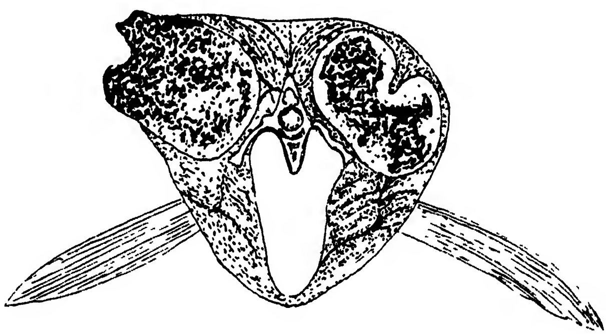


FIG. 305.—*Myxobolus pfeifferi*: SECTION THROUGH THE BODY OF A BARBEL, SHOWING TWO TUMOURS CAUSED BY THE PARASITE. (AFTER KEYSSELITZ, 1908.)

ticular tissue or body space which the species infects. In some cases it is evident that a multiplication of these small forms occurs, and this may take place within the cytoplasm of cells, in the intercellular spaces of the tissues, or in the lumen of the gall bladder or other body cavities. Finally, growth into the large multinucleate plasmodia takes place. It was maintained by Cohn (1896) that *Myxidium lieberkühni*, which infects the gall bladder of fish, was able, in the multinucleate phase, to bud from its surface numerous small uninucleate forms. Laveran and Mesnil (1902*a*) showed that no such budding takes place in this species, and that the formation of numerous short pseudopodia, and the fact that young parasites often become applied to the surface of older ones, are responsible for the misconception. They showed that multiplication takes place by equal or unequal division of the young forms. Kudo (1922*b*) has, however, described a process of internal budding in the case of *Leptotheca ohlmacheri* in the kidney of the frog (Fig. 311).

The behaviour of the spores after ingestion by a new host has been studied by several observers, whose accounts are by no means concordant. In the case of *M. bergense*, a parasite of the gall bladder of the saithe, *Gadus virens*, Auerbach (1910) noted that after entering the duodenum of the fish the polar filaments of the spore were extruded and the two valves of the spore capsule separated. This allowed the binucleate amœboid body to escape. The two nuclei then fused, and the resulting uninucleate amœboid body made its way to the bile ducts, into one of the cells of which

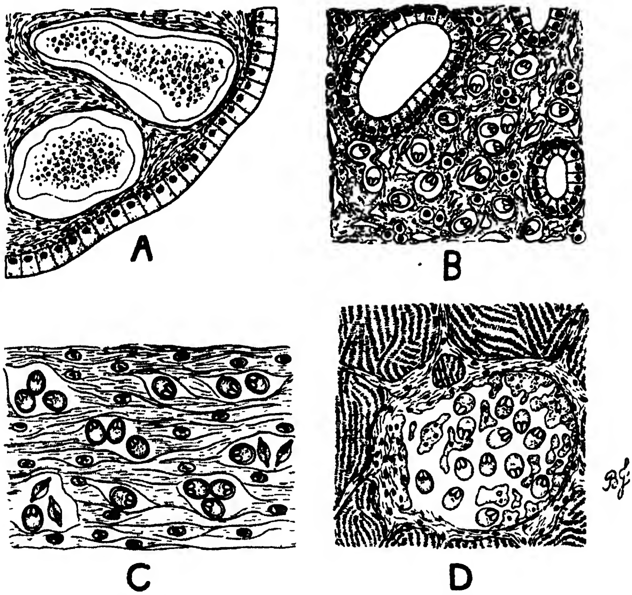


FIG. 306.--*Myxobolus pfeifferi* IN THE TISSUES OF THE BARBEL. (AFTER THÉLOHAN, 1894.)

A. Portion of intestinal wall of the barbel infected with *Myxobolus pfeifferi*.
B. Connective tissue of kidney of barbel infiltrated with *Myxobolus pfeifferi*.
C. Portion of the fibrous tissue shown in A more highly magnified.
D. Muscle fibre of the barbel infected and destroyed by *Myxobolus pfeifferi*. Spores 14 × 10 μ.

it entered. Later it is described as leaving the cell and multiplying by binary fission in the lumen of the bile ducts or gall bladder. The uninucleate amœboid forms then associate in pairs, while the nucleus of one of each pair divides to form two nuclei, one of which is discharged from the cytoplasm. The two cells, one of which has a reduced nucleus, now unite to form a binucleate mass with one large and one small nucleus. Other observers, as, for example, Davis (1916), Georgéwitch (1917), Erdmann (1917), Schuurmans-Stekhoven (1919), and Kudo (1922), working with other species, maintain that such a union does not take place, and that

the binucleate stage is not produced by union of two uninucleate individuals, but by the actual division of the nucleus of the uninucleate form.

The order Myxosporidiida is divided by Doflein (1901) into two suborders, Disporea and Polysporea. The members of the former are parasitic in the body spaces as large amœboid organisms, which may measure as much as 85 by 25 microns. Within each individual a single cell (pansporoblast) is separated in a vacuole. The single uninucleate cell by further development gives rise to two spores, which remain embedded in the cytoplasm of the adult. After their formation the parasite dies and the two spores are liberated. In the Polysporea, which include the majority of the Myxosporidiida, the adult parasite produces a large number of spores. It is these forms which invade the tissues, give rise to large tumours, and often produce an intense infection of the host. In an infected area of tissue, which can only be satisfactorily examined in sections, it is frequently difficult to define the limits of a single parasite, which extends as a reticulum amongst the tissue cells and fibres, producing the condition known as diffuse infiltration (Fig. 306). In such cases the spores, when produced, are scattered amongst the tissue elements, which often show marked hypertrophic changes, though sometimes this does not occur, the individual cells and fibres being little altered in appearance. In those cases in which a fibrous capsule is formed, the multinucleate layer of cytoplasm which lines the capsule appears to be a single parasite.

Kudo (1919) has, however, pointed out that such a division into Disporea and Polysporea is an artificial one, as the number of spores produced by any particular species is by no means as constant as such a classification implies. He maintains that the spore stage is still the only one which affords constant characters by which various genera and species can be identified (Fig. 307).

The spore consists of a shell composed of two valves which are united in a sutural plane like two watch-glasses placed with their rims together. The sutural line may be straight, as in the case of watch-glasses, or it may be more irregularly curved, giving the appearance of an S in side view. The form of the spore varies with the shape of the valves and the presence

1. *Leptotheca informis.*
2. *Myxoproteus cornutus.*
3. *Wardia ovinocua.*
4. *Ceratomyxa spinosa.*
5. *Ceratomyxa truncata.*
6. *Ceratomyxa recurvata.*
7. *Ceratomyxa spherulosa.*
8. *Mitraspora cyprini.*
9. *Mitraspora caudata.*
10. *Chloromyxum leydigi.*
11. *Chloromyxum caudatum.*
12. *Sphærospora rostrata.*
13. *Sinuolinea capsularis.*
14. *Myxidium procerum.*
15. *Myxidium inflatum*
16. *Sphæromyxa balbianii.*
17. *Sphæromyxa incurvata.*
18. *Zschokkella acheilognathi.*
19. *Myxosoma dujardini.*
20. *Myxosoma dujardini.*
21. *Leptospora acuta.*
22. *Myxobolus carassii.*
23. *Hoferellus cyprini.*
24. *Henneguya gurleyi.*
25. *Henneguya psorospermica.*

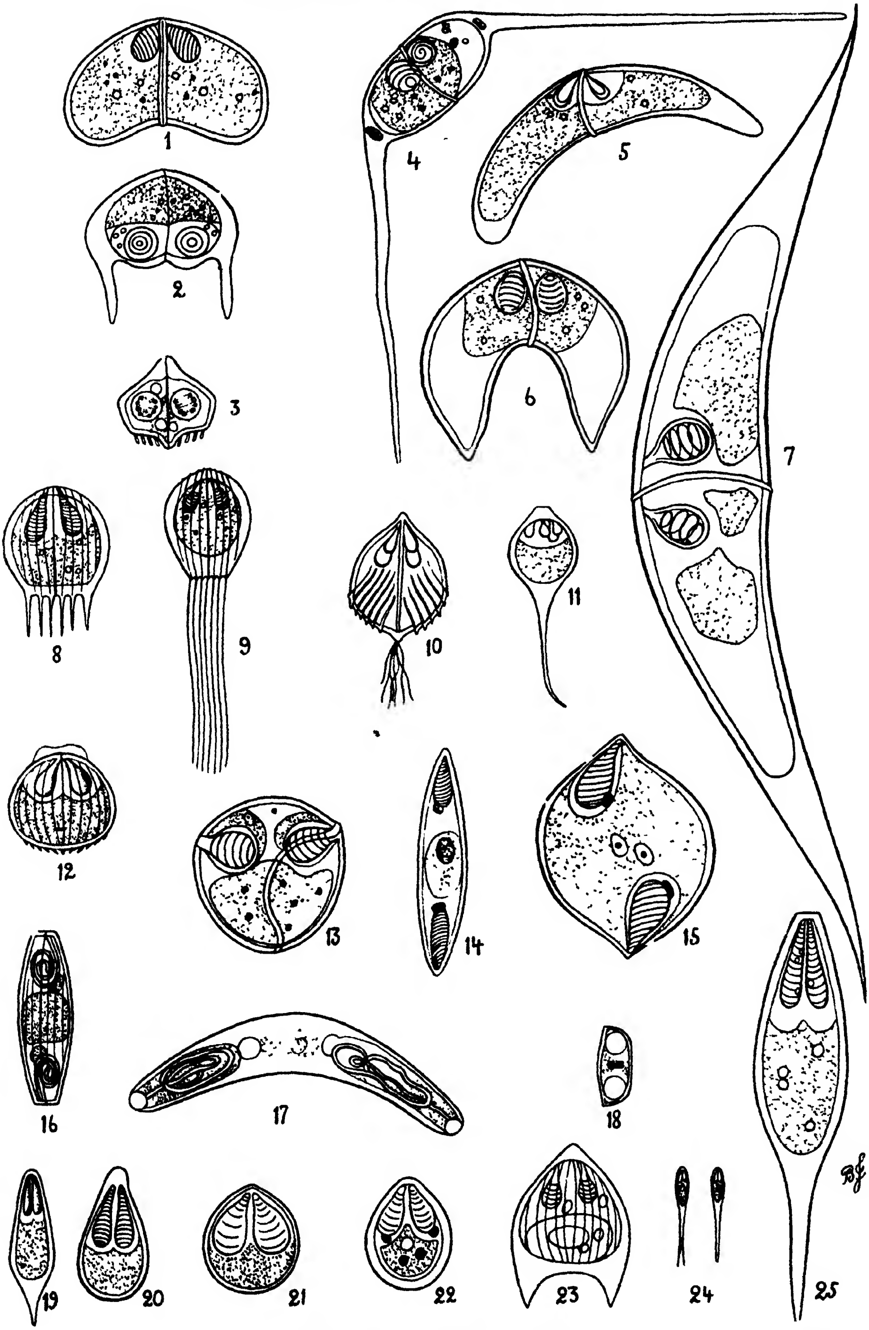


FIG. 307.—SPORES OF MYXOSPORIDIIDA (×1,500). (AFTER KUDO, 1919.)

[*For description see opposite page.*

of accessory appendages (Fig. 307). In *Ceratomyxa* there are lateral appendages, in *Myxoproteus* anterior ones, in *Wardia* a posterior fringe, in *Mitraspora* a posterior filament, in *Hoferellus* a posterior spine, and in *Henneguya* a posterior tail-like process. The surface of the shell or valves is smooth or marked with ridges. Within the shell are the polar capsules, which in most cases are situated at the narrow anterior end of the spore. In the Myxidiidæ there is one at each end of the spore, while in a few species of *Wardia* the polar capsules are central in position. Each polar capsule is spherical or pyriform in shape, and opens to the exterior by a separate pore which is at the anterior end of the spore except in the spores of the Myxidiidæ, in which no distinction between anterior and posterior ends can be made. There are always two polar capsules in the spore, except in *Myxobolus*, which has one, and *Chloromyxum* and *Agarella*, which have four.

Within each polar capsule is a coiled filament, which can be extruded through its pore. The filament is coiled round the long axis of the spore, except in *Sphœromyxa*, in which it is coiled round an axis at right angles to this. The filament is long and thin in all forms except *Sphœromyxa*, in which it is short, thick, and tapering. In addition to the polar capsules, the spore contains the infecting agent in the form of a cytoplasmic body, sometimes called the sporoplasm, containing usually two nuclei, and frequently an iodophilic vacuole filled with glycogenic material.

Subdivision of the Myxosporidiida.

The following classification, taken from Kudo's monograph (1919) on the Myxosporidiida (=Myxosporidia Bütschli, 1881), is based chiefly on the characters of the spores (Fig. 306). It differs in the inclusion of the genus *Agarella* in the family Chloromyxidæ.

1. *Sub-Order:* **Eurysporea** KUDO, 1919.

Largest diameter of the spore at right angles to the sutural plane. One polar capsule on each side of the plane. Sporoplasm with no iodinophilous vacuole. Vegetative form found in body cavity (except in two species). Great majority parasites of marine fish. Monosporous, disporous, and polysporous.

(1) *Family:* CERATOMYXIDÆ Doflein, 1899.

With the characters of the sub-order.

Genus: **Leptotheca** Thélohan, 1895.

Shell valves of spore hemispherical or shortly rounded. Fifteen species. Disporous (seven unknown). Fourteen species in body cavity, one in tissue; all in marine fish. Type species: *Leptotheca agilis* Thélohan.

Genus: **Ceratomyxa** Thélohan, 1892.

Shell valves conical and hollow, attached on the bases; free ends extended, tapering to more or less sharply pointed or rounded ends. Sporoplasm usually does not fill the cavity, but is located asymmetrically in it. Thirty-five species. Disporous (twenty-three species), monosporous and disporous (three species), disporous and polysporous (four species), and unknown (five species). All (except two species in urinary bladder) in the gall bladder of marine fish. Type species: *Ceratomyxa arcuata* Thélohan.

Genus: **Myxoproteus** Doflein, 1898, *emend.* Davis, 1917.

Spores roughly pyramidal; with or without distinct processes from the base of the pyramid. Three species. Disporous (one species unknown). All in urinary bladder of marine fish. Type species: *Myxoproteus ambiguus* (Thélohan) Doflein.

Genus: **Wardia** Kudo, 1919.

Spore form of isosceles triangle with two convex sides. Oval in profile. Surface of shell with fine ridges which turn into fringe-like processes at the posterior end. The polar capsules, large and perfectly spherical, situated at the central portion of the spore, opening at the anterior tip. Two species. Polysporous (one species unknown). Tissue parasite (one species) of fresh-water fish and amphibia, both found in Illinois, U.S.A. Type species: *Wardia ovinocua* Kudo, 1919.

Genus: **Mitraspora** Fujita, 1912, *emend.* Kudo, 1919.

Spores spherical or ovoidal. Two polar capsules pyriform, one situated on each side of the sutural plane. Shell longitudinally striated, with or without long and fine filaments projecting posteriorly in a row at right angles to the sutural plane at the posterior side. Three species. Disporous and polysporous. All found in kidney of fresh-water fish. Type species: *Mitraspora cyprini* Fujita.

2. *Sub-Order:* **Sphærosporea** KUDO, 1919.

Spores spherical or subspherical, with two to four polar capsules. Sporoplasm without iodinophilous vacuole. Vegetative form found in body cavity and tissue. Monosporous, disporous, and polysporous. Parasites of marine and fresh-water fish and amphibia.

(1) *Family:* CHLOROMYXIDÆ Thélohan, 1892.

Spores with four polar capsules. Monosporous, disporous, and polysporous.

Genus: **Chloromyxum** Mingazzini, 1890.

With the characters of the family. Spores without posterior tail-like prolongations. Twenty-two species. Eighteen in body cavity, four in tissue. Seven from marine and twelve from fresh-water fish, two in amphibia, one in insect. Type species: *Chloromyxum leydigi* Mingazzini.

Genus: **Agarella** Dunkerly, 1915.

Spores prolonged at posterior end into two processes. Only species *Agarella gracilis* Dunkerly from testis of *Lepidosiren paradoxa.*

(2) *Family:* SPHÆROSPORIDÆ Davis, 1917.

Spores with two polar capsules. Monosporous, disporous, and polysporous.

Genus: **Sphærospora** Thélohan, 1892.

Spores with two polar capsules. Monosporous, disporous, and polysporous. Ten species. Body cavity and tissue. Five from fresh-water and five from marine fish. Type species: *Sphærospora divergens* Thélohan.

Genus: **Sinuolinea** Davis, 1917.

Spores with or without lateral processes. Two polar capsules spherical. Sutural line sinuous. Five species. Disporous and polysporous. In the urinary bladder of marine fish. Type species: *Sinuolinea dimorpha* Davis.

3. *Sub-Order:* **Platysporea** KUDO, 1919.

Sutural plane of the spore coincides with or forms an acute angle with the longest diameter. One or two polar capsules. Sporoplasm with or without an iodinophilous vacuole.

(1) *Family:* MYXIDIIDÆ Thélohan, 1892.

Two polar capsules, one at each end. Sporoplasm without any iodinophilous vacuole. Spores fusiform.

Genus: **Myxidium** Bütschli, 1882.

Spores more or less regularly fusiform, with pointed or rounded ends. Polar filaments long and fine. Twenty-six species. Monosporous, disporous, and polysporous. Twenty-two in body cavity, four in tissue. Fifteen in marine and six in fresh-water fish, two in fishes from both waters, and three in reptilia. Type species: *Myxidium lieberkühni* Bütschli.

Genus: **Sphæromyxa** Thélohan, 1892.

Spores fusiform, with truncated ends. Polar filament short and thick. Trophozoites large and disc-shaped. Seven species. Polysporous (two unknown). Six in body cavity, six in marine fish, one in amphibia. Type species: *Sphæromyxa balbianii* Thélohan.

Genus: **Zschokkella** Auerbach, 1910.

Spores semicircular in front view, pointed at ends. Polar capsules large and spherical, opening on the flat edge near the tips. Sutural line usually curved in **S** form. Four species. Monosporous, disporous, and polysporous. Body cavity. Two from marine and two from fresh-water fish. Type species: *Zschokkella hildæ* Auerbach.

(2) *Family:* MYXOSOMATIDÆ Poche, 1913.

Two polar capsules at the anterior end. Sporoplasm without iodinophilous vacuole.

Genus: **Myxosoma** Thélohan, 1892.

Spores ovoidal, flattened, and more or less elongated. Three species. Polysporous. Tissue parasites. Two in fresh-water and one in marine fish. Type species: *Myxosoma dujardini* Thélohan.

Genus: **Lentospora** Plehn, 1905.

Spores similar to Myxobolus in form. Sporoplasm without any iodinophilous vacuole. Six species. Disporous and polysporous (two unknown). One in marine and three in fresh-water fish, two from fishes in both waters. Type species: *Lentospora cerebralis* (Hofer) Plehn.

(3) *Family:* MYXOBOLIDÆ Thélohan, 1892.

Spores with one or two polar capsules at the anterior end, with or without posterior processes. Sporoplasm with an iodinophilous vacuole. Majority polysporous in fresh-water fishes.

Genus: **Myxobolus** Bütschli, 1882.

Spores ovoidal or ellipsoidal; flattened. One or two polar capsules at the anterior end. Shell without posterior process. Sixty-three species. Polysporous (nine species unknown). Fifty-nine species in tissue; four unknown. Five in marine and fifty-six in fresh-water fish, one in annelid, and one in amphibia. Type species: *Myxobolus mülleri* Bütschli.

Genus: **Henneguya** Thélohan, 1892.

Spores more or less globular or ovoidal. Two polar capsules at the anterior end. Posterior end of the shell valves prolonged into more or less extended processes, which unite and form a tail in the median line. Thirty-two species. Polysporous, disporous, and monosporous. Twenty-eight species in tissue and four in body cavity. In fresh-water fish, except one. Type species: *Henneguya psorospermica* Thélohan.

Genus: **Hoferellus** Berg, 1898.

Spores pyramidal, with two posterior processes from the lateral faces. One species. Polysporous. Tissue and body cavity of fresh-water fish. Type and only species: *Hoferellus cyprini* Doflein.

DETAILED DESCRIPTION OF CERTAIN SPECIES.

Myxobolus pfeifferi Thélohan, 1894. — This organism is a common parasite of the barbel, *Barbus fluviatilis*. It infests all the tissues of the body, including the skin and gills, on which it gives rise to nodules and tumours, which may be of large dimensions. In the infected tissues the plasmodia are closely intermingled with the host cells, so that often there is difficulty in defining the limits of the parasite. The infected area of tissue is frequently enclosed by a fibrous capsule formed by the host, and in this the parasite in the form of a multinucleate plasmodium continues its development (Fig. 305). Spores are continuously being formed in the cytoplasm of the parasite. Eventually the parasite dies, and all that remains is a fibrous nodule which, on section, is seen to contain many spores in the interstices of the tissue. Spore formation has been studied by Keysselitz (1908). In the plasmodium, one of the nuclei becomes separated with some cytoplasm as a cell, which Keysselitz calls the propagative cell (Fig. 308). After unequal nuclear division a large and small cell are produced. Two such couples become associated, and the aggregation of the two large and two small cells proceeds to the development of two spores. The two small cells spread over the surface of the two large cells to form an envelope, while the nuclei of the two large cells multiply till a total of twelve are present. The cytoplasm of the two large cells may now unite. Of the twelve nuclei, four, which are gamete nuclei, become centrally placed, while eight take up a peripheral position. If the cytoplasm has united division takes place, so that two bodies are produced, each with two centrally placed gamete nuclei and four peripheral nuclei. Each of these cells with six nuclei develops into a spore. Two of the peripheral nuclei form the two valves of the spore, while two give rise to

the two polar capsules. The two gamete nuclei come to lie in a cytoplasmic body at the posterior part of the spore. Finally, the two gamete nuclei fuse. Apparently, the spores escape into the water and are ingested by fish. The amœboid body presumably escapes from the spore and

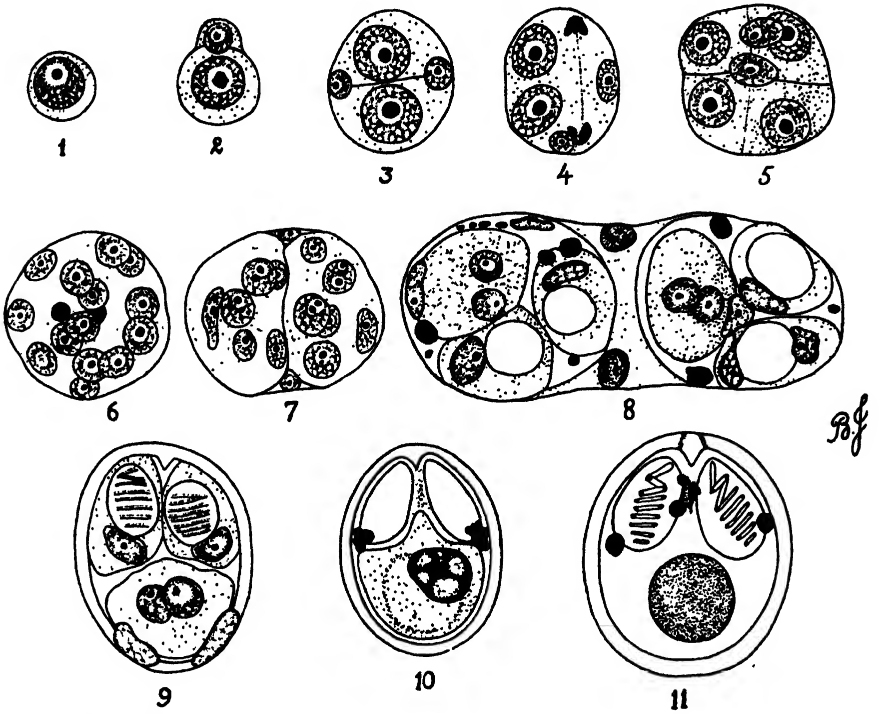


FIG. 308.—DEVELOPMENT OF THE SPORES OF *Myxobolus pfeifferi* FROM THE PANSPOROBLAST (×2,500). (AFTER KEYSSELITZ, 1908.)

1. Single propagative cell formed from the multinucleate plasmodium.
2. Division to form one large and one small cell.
3. Association of two pairs to give a group of two large and two small cells.
4-5. Formation of six-cell stage. Each small cell which does not multiply tends to spread a covering over its own sister cells.
6. Stage with fourteen nuclei, two of which are the nuclei of the original small cells.
7. Division into two bodies, each with six nuclei, while the nuclei of the small cells take up a position at the angles between them.
8. Each body now divides into three cells, two of which, with single nuclei and vacuoles, form the polar capsules, one with two nuclei the infective agent, while two nuclei become peripherally arranged and form, together with some cytoplasm, the valves of the spore.
9. More advanced stage of one of the developing spores shown in 7.
10-11. Fully developed spores.

finds its way to the tissues of the fish, in which it develops into the multinucleate plasmodium. As is to be expected, the tracing of this part of the development is beset with many difficulties. Other observers, such as Schuurmans-Stekhoven (1919), state that there is no syngamy.

Sphæromyxa sabrazesi Laveran and Mesnil, 1900.—This parasite was discovered in the gall bladder of the sea-horse, *Hippocampus brevirostris*, by Laveran and Mesnil (1900). Schröder (1907, 1910) studied its development in the gall bladder of *H. guttulatus*. It lives in the gall bladder and larger bile ducts as a more or less circular disc of cytoplasm, which may reach a diameter of half a centimetre. There is a definite hyaline ectoplasm and a much vacuolated endoplasm, in which numerous refractile granules are embedded. It is probable that infection is com-

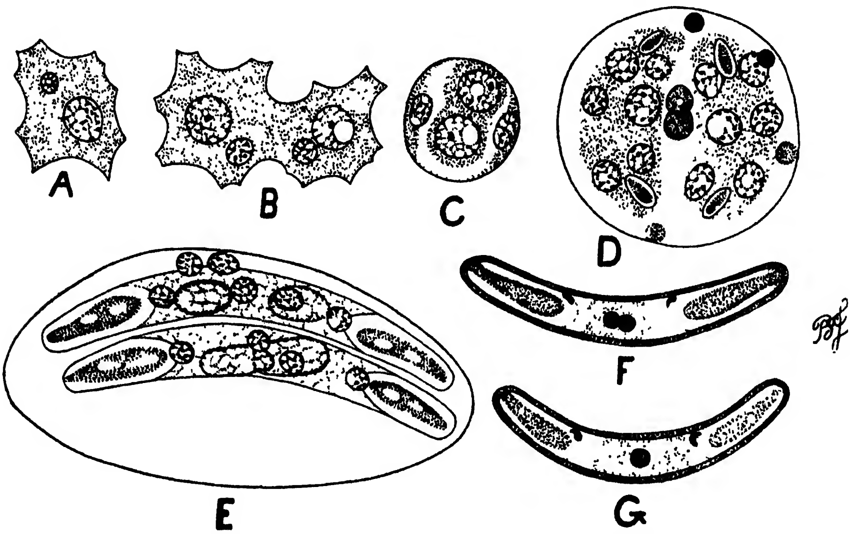


FIG. 309.—SPORE FORMATION IN *Sphæromyxa sabrazesi* (× *ca.* 1,500). (AFTER SCHRÖDER, 1907 AND 1910.)

A. Propagative cell with two nuclei. B. Union of two propagative cells.
C. Cell with four nuclei, two small nuclei of envelope cells, and two large nuclei, which contribute to the formation of two spores.
D. Division of the cell after nuclear multiplication into two spore-forming bodies. Each contains six nuclei and two commencing polar capsules. At the centre are two residual nuclei. E. Two spores nearing completion.
F. Complete spore before union of two nuclei in the sporoplasm.
G. Two nuclei of sporoplasm have united.

menced by the small uninucleate amœboid body which, escaping from the spore in the intestine of the fish, invades the bile ducts. By nuclear multiplication and growth of the cytoplasm the large plasmodia are produced. Spore formation commences by the separation of one of the nuclei with some cytoplasm as a small cell, which remains in the cytoplasm of the parent (Fig. 309). The nucleus of this cell divides into a large and a small nucleus, and, as in the case of *Myxobolus pfeifferi*, two such binucleate cells become fused into a single quadrinucleate cell, which contains two large and two small nuclei. This cell gives rise by further

development to two spores. The process of spore formation is very similar to that of *M. pfeifferi*, but the spores are very different in character. The infective amœboid body in the fully-formed spore has at first two nuclei, but these fuse, so that the final infective agent has a single nucleus. During spore formation the parasite continues to increase in size, while nuclear multiplication is going on. Each parasite produces a large number of spores, which in any individual are in various stages of development.

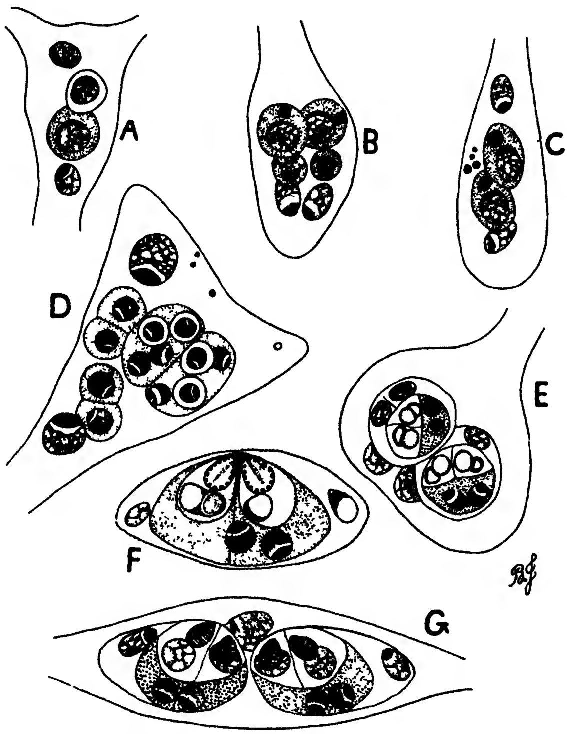


FIG. 310.—*Ceratomyxa drepanopsettæ* (× *ca.* 700). (AFTER AWERINZEW, 1909.)

A. Parasite with two vegetative nuclei and a macrogametocyte and microgametocyte.
B. Similar form in which the gametocytes have divided into two macrogametes and two microgametes. C. Similar form after conjugation of the gametes.
D. Each zygote has given rise to six cells. E. Each group of six cells is producing a spore.
F. Single spore nearing maturity. G. More advanced stage of development of two spores.

Ceratomyxa drepanopsettæ Awerinzew, 1909.—This parasite was discovered by Awerinzew (1909) in the gall bladder of the plaice (*Drepanopsetta platessoides*), where it lives as an amœboid organism. At first the trophozoite has two nuclei, and it was concluded by Awerinzew that this stage resulted from nuclear division of a uninucleate form. In view of the subsequent conjugation process, Minchin (1912) concluded that it was more probable that union of two uninucleate forms had taken place.

Each nucleus now divides unequally, so that two small and two large nuclei are present (Fig. 310). The small ones are vegetative and the large ones generative nuclei. The generative nuclei become separated in the cytoplasm of the parent as a large and a small cell, which may be regarded as female and male gametocytes. Each of the gametocytes divides to form two large female gametes and two small male gametes, while the two vegetative nuclei remain unchanged. Conjugation between male and female gametes takes place to form two zygotes, so that the parasite again reaches a quadrinucleate stage in which its cytoplasm contains two vegetative nuclei and two zygotes, each with a single nucleus. Each zygote now proceeds to the formation of a spore. A number of cells is produced, two of which give rise to the two valves of the spore, two to the two polar capsules, and one to the infective amœboid body. After each zygote has formed a spore the parasite dies and degenerates, the spores being liberated. Thus each parasite produces only two spores. It was Myxosporidiida of this type that Doflein (1901) grouped under the heading Disporea.

Leptotheca ohlmacheri (Gurley, 1893).—This parasite was first observed by Ohlmacher (1893) in the kidney tubules of *Bufo lentiginosus*. It was studied by Whinery (1893) and Gurley (1893, 1894). The latter observer named it *Chloromyxum ohlmacheri*, while Thélohan (1895) gave the name *Leptotheca ranæ* to a form in the kidneys of *Rana esculenta* and *R. temporaria*. Labbé (1899) placed Ohlmacher's parasite in the genus *Leptotheca* as *L. ohlmacheri*, and came to the conclusion that it was identical with *L. ranæ*. The parasite has more recently been studied by Kudo (1922*b*) in *R. clamitans* and *R. pipiens* in America.

According to Kudo, the spore contains two uninucleate amœboid bodies, and when it is placed in gastric juice or weak pepsin hydrochloric acid, the amœboid bodies show slow movements. The polar filaments are extruded from the capsules, and finally the valves of the spore separate (Fig. 311). By this time the two amœboid bodies have united into a single binucleate form, which escapes from the spore. It is probable that the two nuclei fuse, for the earliest stages found in the kidney tubules contain a single nucleus. There was no evidence of any intracellular stage, the whole development appearing to take place in the lumen of the tubules. It seems probable that multiplication of these forms by binary fission takes place for some time, after which growth into the adult spore-forming parasite occurs.

The single nucleus divides into two nuclei of equal size, which, though at first alike, soon become different in appearance, so that a vegetative nucleus can be distinguished from a generative one. The latter quickly divides again, so that a trinucleate parasite is produced. This contains

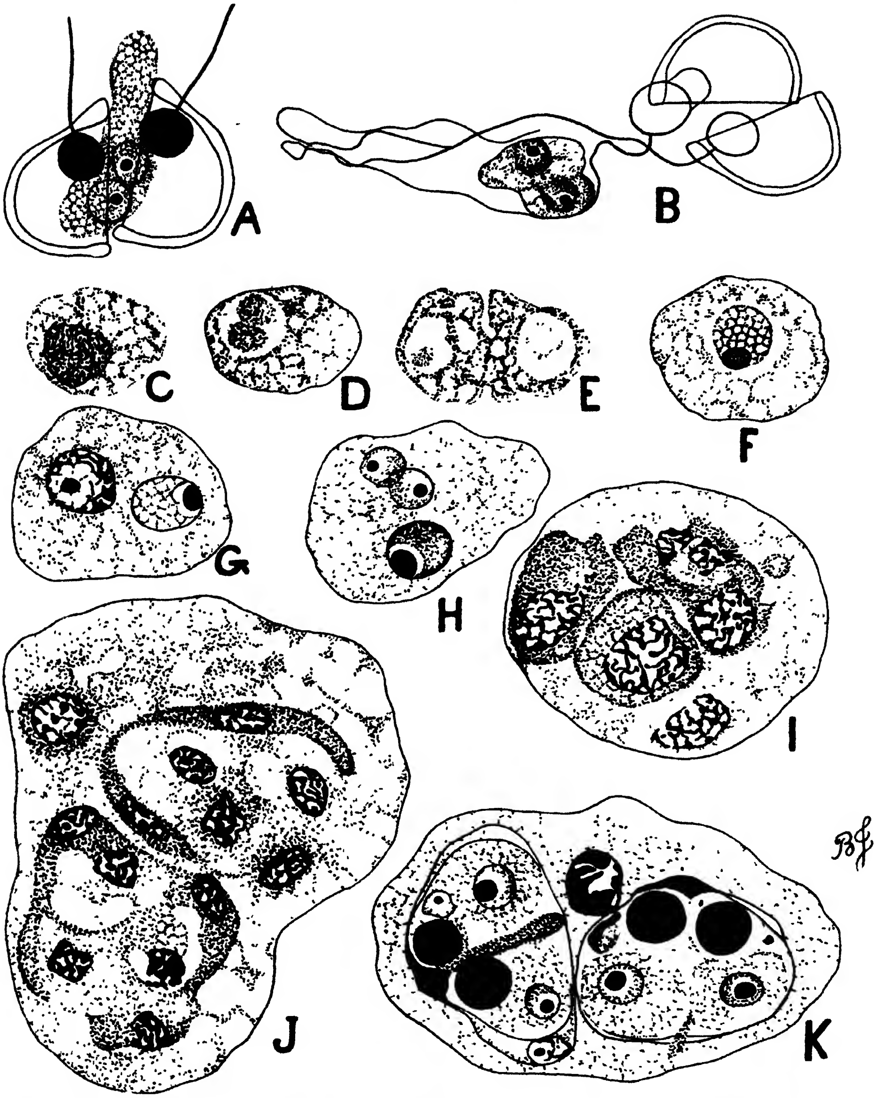


FIG. 311.—*Leptotheca ohlmacheri*, PARASITIC IN THE KIDNEY TUBULES OF THE FROG (× 2,350). (AFTER KUDO, 1923).

A. Separation of values of the spore, extrusion of polar filaments, and escape of binucleate amœboid body under the action of pepsin hydrochloric acid.
B. Later stage of escape of amœboid body under action of gastric juice.
C. Amœboid infective body in which the two nuclei have united.
D, E. Multiplication by binary fission. F. Fully grown uninucleate form.
G. Nucleus has divided into vegetative and generative nuclei.
H. The generative nucleus has divided to form the typical trinucleated form.
I. Form with two generative nuclei, one vegetative nucleus, and a bud containing also a smaller vegetative nucleus and two smaller generative nuclei.
J. Form with one vegetative nucleus and two areas, each with six generative nuclei, which will give rise to two spores.
K. Form with one vegetative nucleus and two spores nearing the completion of their development.

one vegetative nucleus and two generative nuclei. By a process of gemmation or budding trinucleate individuals may be separated. In this process the vegetative nucleus divides into two. One of these divides again to form a vegetative nucleus and a generative nucleus. The latter again divides to form two generative nuclei. Round the group of these three nuclei cytoplasm concentrates and the trinucleate bud is separated. Apparently this process can be repeated several times, so that the number of trinucleate parasites in the kidney tubules is greatly increased in number.

Spore formation takes place in the trinucleate forms, each generative nucleus giving rise to one spore. The generative nucleus divides to form two nuclei, and these again to form four. Of these four, two are devoted to the formation of the two valves of the spore, one divides to form two nuclei which give rise to the two polar capsules, while the fourth divides to form the nuclei of the two infective amœboid bodies which occur in the fully-formed spore. Each generative nucleus of the original trinucleate individual thus gives rise to six nuclei, so that a parasite in which two spores are developing simultaneously, which is not always the case, will have one vegetative nucleus and twelve generative nuclei, each group of six generative nuclei being destined to form one spore.

B. *Order:* MICROSPORIDIIDA Labbé, 1899.

The parasites included in this order produce small spores, which are frequently less than 5 microns in length. The spores often resemble yeasts or bacilli, but possess one or, exceptionally, two polar capsules from which, after treatment with certain reagents or under pressure, exceedingly long filaments are extruded (Fig. 30). The latter may reach a length of 500 microns or more. The organisms occur as intracellular amœboid parasites (Fig. 312). As multiplication takes place, the parasitized cells often become hypertrophied in a remarkable manner. In the case of some hosts, only special organs are attacked, but in others, as in the silkworm disease, the whole body is overrun by the parasites. The ova may become infected, with the result that the parasites pass from the parent to the offspring. After multiplication in the amœboid phase has gone on for some time, certain spherical uninucleate forms (pansporoblasts or sporonts) undergo a complicated development to produce the characteristic spores. The spores produced by each pansporoblast vary in number from one to sixteen or more according to the particular genus or species.

The Microsporidiida are found commonly in the intestinal epithelium and other tissues of the aquatic larvæ of insects. They occur also in certain vertebrates such as fish, in which tumour-like nodules may be

formed in the muscles or skin. The infection can be recognized by the presence of the numerous spores in the teased-out tissues. In sections it

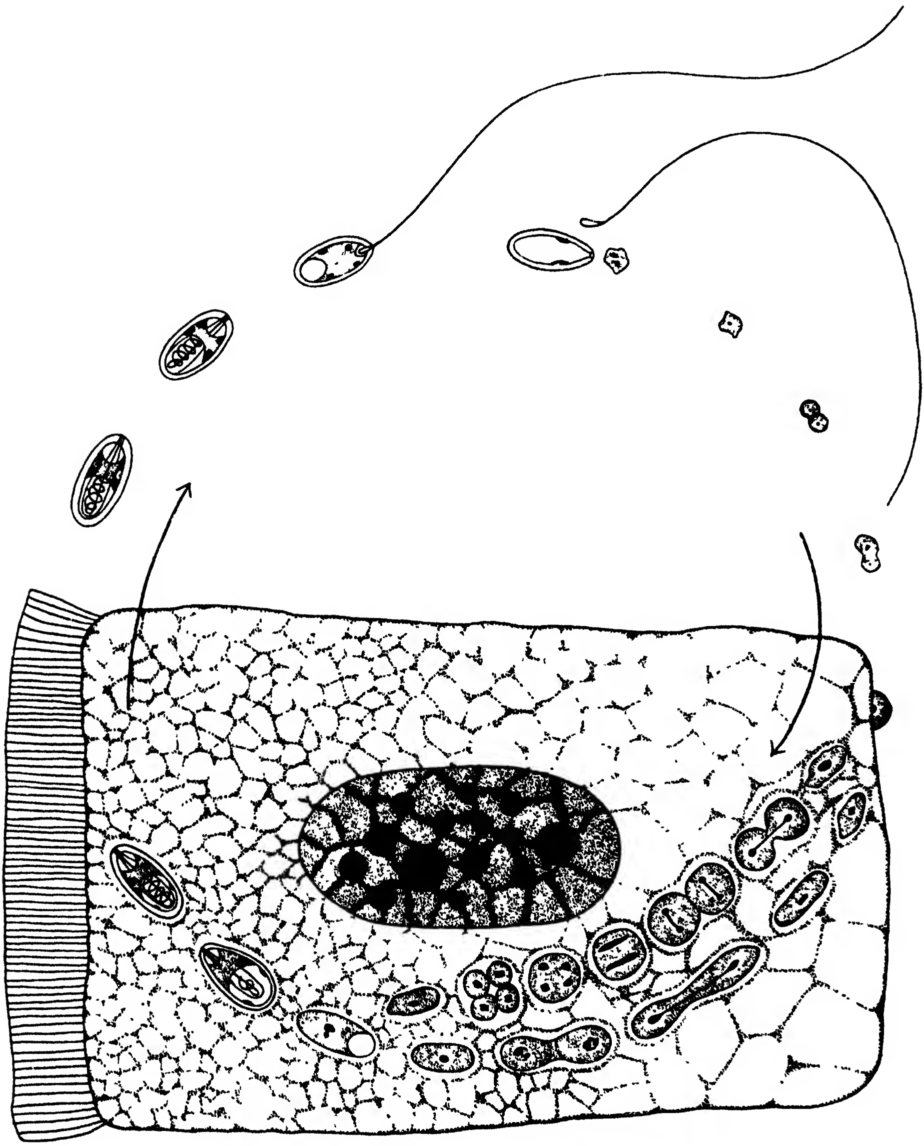


FIG. 312.—DIAGRAM OF LIFE-CYCLE OF *Nosema bombycis* IN INTESTINAL EPITHELIUM OF SILKWORM (× *ca.* 3,000). (AFTER STEMPELL, 1909.)

can be seen that the infected cells of the intestine or other tissues are hypertrophied and filled with numerous minute, rounded, or amœboid

cytoplasmic bodies containing one or more nuclei, and multiplying by binary fission or multiple segmentation. Large numbers of spores also occur in the cells, and these often have the appearance of yeasts, cocci, or bacilli, from which they may be difficult to distinguish, unless there is an opportunity of causing extrusion of the polar filament. The presence of the polar capsule and the extrusion of its filament can be rendered evident by treating the spores with irritating fluids such as dilute acid, iodine solution, or perhydrol, or by pressure between slide and cover-glass. In the fresh condition, the filaments are best seen by dark ground illumination (Fig. 30). They may be stained by the silver nitrate methods employed for demonstrating spirochætes. Owing to the small size of the spores of the majority of Microsporidiida, their detailed structure is difficult to make out, while the varying effect of different fixatives accounts for the great diversity of the accounts which have been given. Stempell (1909) described the spore of *Nosema bombycis* (Fig. 312). He believed that the polar capsule was an elongated body occupying the length of the spore, and that the infective agent was in the form of an equatorial band of cytoplasm surrounding the polar capsule in the space between it and the spore wall. This cytoplasm was described as containing four nuclei. Many observers have adopted Stempell's views regarding the structure of the spore. Schuberg (1910), working with *Plistophora longifilis*, stated that the polar filament was coiled on the inner surface of the spore wall, and that the infective agent was in the form of a circular band of cytoplasm containing a single nucleus. He maintained that a definite polar capsule did not exist. Léger and Hesse (1916*a*), in the case of the spores of *P. macrospora*, *N. bombycis*, and other forms, described the polar capsule as a large sac-like body occupying the greater part of the interior of the spore, and the infective agent as a small mass of cytoplasm in a clear space at the posterior end of the spore. The polar filament was coiled within the polar capsule. The band of cytoplasm described by other observers appeared to be nothing more than the retracted and distorted polar capsule itself, and the supposed nuclei in it optical cross-sections of the coiled filament. Kudo (1920) found that the spores of *Stempellia magna*, which, on account of their large size, were very suitable objects of study, were constructed as Léger and Hesse maintained (Fig. 313). The spores of the genus *Mrazekia*, as described by Léger and Hesse (1916), are formed on the same plan, with the exception that the proximal part of the polar filament is thickened as an axial manubrium (Fig. 317). As regards the minute structure of the smallest spores (*Cocconema*, *Toxonema*, *Spirillonema*), nothing is known (Fig. 318).

It is probably safe to assume that the spores of Microsporidiida have a large polar capsule occupying the bulk of the interior of the spore, and

that the infective agent, containing one or two nuclei, lies behind the polar capsule in the clear space at the posterior part of the spore. The envelope of the spore consists, in some cases at least, of two valves. The development of the spore from the single cell (sporoblast) which gives rise to it appears to be a very complicated one. A number of cells are formed, as in the development of the spores of Myxosporidiida, and some of these give rise to the outer covering of the spore; others form the polar capsule and infective amœboid body.

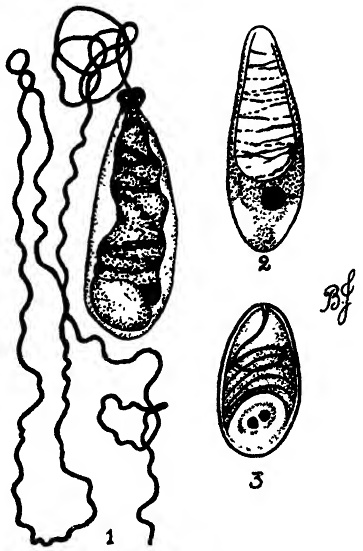


FIG. 313.—STRUCTURE OF MICROSPORIDIAN SPORES. (1 AND 2, AFTER KUDO, 1920; 3, AFTER LÉGER AND HESSE, 1916.)

1-2. Spore of *Stempellia magna* ($\times 2,360$).
1. Extruded filament, polar capsule, and infective body.
2. Same before extrusion of filament.
3. Spore of *Plistophora macrospora* ($\times 2,500$). Polar capsule with coiled filament and infective body with two nuclei.

Subdivision of the Microsporidiida.

Doflein (1901) classified the Microsporidiida on the basis of the number of spores produced by each pansporoblast, but Léger and Hesse (1922*a*) point out that many forms are far from constant in the number of spores produced, a fact previously noted by Chatton and Krempf (1911). It is maintained that the only constant feature on which a classification can be based is the character of the spore itself, and, as in Kudo's classification of the Myxosporidiida, they propose a system which has the character of the spore as its basis. They divide the Microsporidiida into two groups – the Monocnidea, which have spores with one polar capsule; and the Dicnidea, with spores with two polar capsules. The former appear to be of two types. In the one the parasite is a multinucleate cytoplasmic body, which is constantly increasing in size, and when developing often includes the nuclei of tissue cells. As in the Myxosporidiida, certain uninucleated cells (pansporoblasts or sporonts) become separated in vacuoles in the parent cytoplasm, and these give rise to spores (Fig. 304, D). Parasites of this type are called Polysporogenea (family Glugeidæ) by Doflein to distinguish them from those of the second type, Oligosporogenea (family Nosematidæ), which in the vegetative stage are uninucleate bodies multiplying by binary fission or schizogony. Finally, as in the Polysporogenea, pansporoblasts are formed, and these give rise to a varying number of spores (one to sixteen or more). It must be admitted, however, that

there is some doubt as to the exact method of spore formation in the Polysporogenea, which in many cases give rise to tumour-like structures in fish, in which the parasites and the host tissues are so closely intermingled (diffuse infiltration) that the details of development are difficult to follow. The Oligosporogenea are more readily studied, as the small uninucleate parasites are scattered through the cytoplasm of cells.

The following classification of the order Microsporidiida is based on that suggested by Léger and Hesse:

1. *Sub-Order:* **Monocnidea** Léger and Hesse, 1922.

The spore, which varies in shape, has only a single polar capsule.

(1) *Family:* GLUGEIDÆ Gurley, 1893.

The spores, ovoid or pyriform in shape, are developed from pansporoblasts formed in vacuolic spaces in the cytoplasm of the parasite, which continues to grow and produce more nuclei as spore formation is proceeding. Each pansporoblast gives rise to two sporoblasts, and finally to two spores.

Genus: **Glugea** Thélohan, 1891.

(2) *Family:* NOSEMATIDÆ Labbé, 1899.

The spores, which are ovoid or pyriform in shape, are developed from uninucleate rounded bodies which are the products of multiple or binary fission of the vegetative forms. Each uninucleate body, which is a pansporoblast, gives rise to a varying number of spores, which may or may not be enclosed in a capsule.

Genus: **Nosema** Nägeli, 1857.

Each pansporoblast gives rise to a single spore.

Genus: **Perezia** Léger and Duboscq, 1909.

Each pansporoblast gives rise to two spores.

Genus: **Gurleya** Doflein, 1898.

The spores are elongated, being broad at one end and somewhat tapering at the other. Each pansporoblast gives rise to four spores.

Genus: **Thelohania** Henneguy, 1892.

Each pansporoblast typically gives rise to eight spores, but sometimes only four or as many as sixteen are formed.

Genus: **Stempellia** Léger and Hesse, 1910.

Each pansporoblast gives rise to one, two, four, or eight spores, which vary in length from 2 to 6 microns. The smallest spores occur when eight are formed, and the largest when there is only one.

Genus: **Duboscqia** Pérez, 1908.

Each pansporoblast gives rise to sixteen spores.

Genus: **Plistophora** Gurley, 1893.

Each pansporoblast gives rise to many spores (more than sixteen).

(3) *Family:* COCCONEMIDÆ Léger and Hesse, 1921.

The spores are spherical and resemble cocci.

Genus: **Cocconema** Léger and Hesse, 1921.

(4) *Family:* MRAZEKIIDÆ Léger and Hesse, 1922.

The spores are cylindrical, and are either straight, spiral, or curved. They resemble bacilli, vibrios, or spirilla.

Genus: **Mrazekia** Léger and Hesse, 1916.

The spores are cylindrical, like bacilli. Each has an axial manubrium, which can be extruded from one end of the spore. The polar filament is attached to the end of the manubrium. Each pansporoblast gives rise to one or more spores.

Genus: **Octosporea** Flu, 1911.

The spores are slightly arched and cylindrical, like bacilli, but there is no axial manubrium. Each pansporoblast gives rise to eight or sixteen spores.

Genus: **Toxonema** Léger and Hesse, 1922.

The spore is arched and resembles a vibrio. The pansporoblast gives rise to eight spores.

Genus: **Spirillonema** (=**Spironema** Léger and Hesse, 1922).

The spores are spiral and resemble spirilla. The pansporoblast gives rise to eight spores.

2. *Sub-Order:* **Dicnidea** LÉGER AND HESSE, 1922.

The spore is oval in outline, and possesses two polar capsules, one at each end of the spore.

Family: TELOMYXIDÆ Léger and Hesse, 1910.

Genus: **Telomyxa** Léger and Hesse, 1910.

DETAILED DESCRIPTION OF CERTAIN GENERA AND SPECIES.

Genus: **Glugea** Thélohan, 1891.

The members of this genus are typically parasites of fish, but they occur also in reptiles, frogs, and worms. Their characteristic feature is that they occur as multinucleate plasmodia, as a rule embedded in or infiltrating the tissues. The ovoid spores are produced from pansporoblasts, which are separated in vacuoles in the multinucleate plasmodium. Sometimes they occur free in the body-cavity spaces. In this respect they resemble the Myxosporidiida (Fig. 304, D).

Glugea anomala (Moniez, 1887).—This is a parasite of the tissues and organs of various fresh-water fish, chiefly the sticklebacks (*Gasterosteus*), on the skin of which it gives rise to white nodules. On section such a nodule is seen to have a fibrous capsule, within which is a multinucleate cytoplasmic body. The central part of the nodule is occupied by numerous ovoid spores, and these are also present in vacuoles in the peripheral cytoplasmic part. There are also present a number of large nuclei, which appear to be the nuclei of the tissue cells which have been almost completely destroyed. The spores are ovoid, and measure, as a rule, from 4 to 4·5 by 3 microns (Fig. 381, I). The polar filament may be 150 microns long.

Genus: **Nosema** Nägeli, 1857.

This genus includes Microsporidiida, which in the vegetative phase resemble the members of the genus *Thelohania*. The uninucleate pansporoblast by a complicated process of development gives rise, however, to a single spore.

Nosema bombycis Nägeli, 1857.—This parasite, which is the best-known member of the genus, gives rise to the notorious silkworm disease. Its life-history was studied by Stempell (1909). The infection is commenced by the small amœboid body which escapes from the spore after its ingestion by a silkworm *Bombyx mori* (Figs. 312 and 314). It multiplies in the intestine. The resulting parasites, which are uninucleate, pass between the epithelial cells into the hæmocœle space, and thence into the various tissues of the body, including the ovary. These stages were called planonts by Stempell. They eventually enter the cytoplasm of cells and become meronts, which multiply by binary fission, gemmation, or schizogony. The products of this multiplication are often arranged in rows like a string of beads. After the cytoplasm of the cell is exhausted, the uninucleate forms become transformed into spores. Four nuclei are formed in each, and two of these with some of the cytoplasm form the spore capsule, while, of the two remaining, one takes part in the formation of the terminal

polar capsule, and the other becomes the nucleus of the infective amœboid form or sporozoite, which escapes from the spore when it is taken into the intestine of a new host. The spores are so minute and the capsule so thick that the details of the development are exceedingly difficult to follow. Another form, *Nosema apis* Zander, 1909, with spores measuring 4·6 to 6·4 by 2·5 to 3·4 microns, is supposed to be the cause of bee disease (Fig. 30),

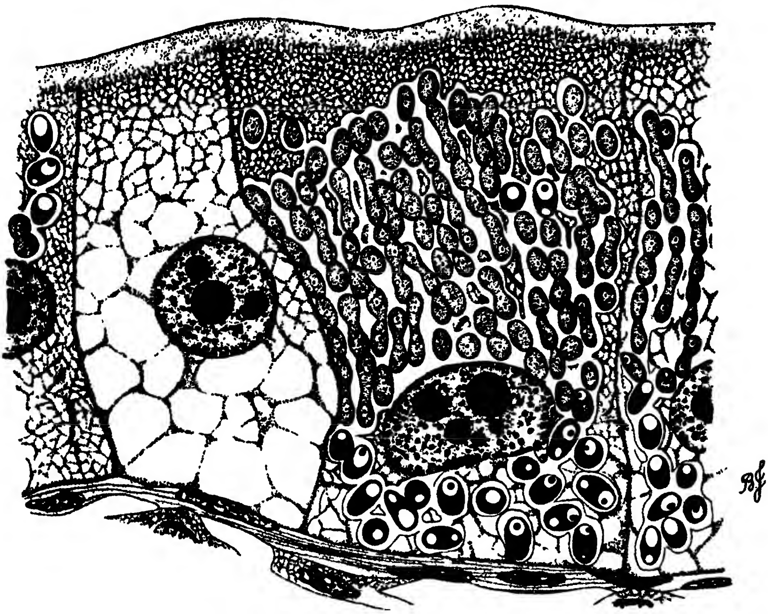


FIG. 314.—*Nosema bombycis*: DEVELOPMENTAL STAGES AND SPORE FORMATION IN INTESTINAL EPITHELIAL CELLS OF SILKWORM (× *ca.* 2,000). (AFTER STEMPELL, 1909.)

while *N. frenzelinæ* Léger and Duboscq, 1909, parasitizes a gregarine (*Frenzelina conformis*), which is itself parasitic in the crab, *Pachygrapsus marmoratus.*

Genus: **Gurleya** Doflein, 1897.

The members of this genus have elongate spores, which are broader at one end than at the other.

Gurleya francottei Léger and Duboscq, 1909.—This organism is parasitic in the epithelium of larvæ of *Ptychoptera contaminata.* The pansporoblast gives rise to four spores, which are radially arranged (Fig. 315).

Genus: **Thelohania** Henneguy, 1892.

The members of this genus occur as minute parasites in the cytoplasm of cells of aquatic invertebrates. There is little tendency to the production of multinucleate plasmodia. Multiplication is usually by binary

fission, but sometimes division into uninucleate forms does not take place till a larger number of nuclei is present. Eventually, a uninucleate form becomes a pansporoblast, and produces typically eight ovoid spores which

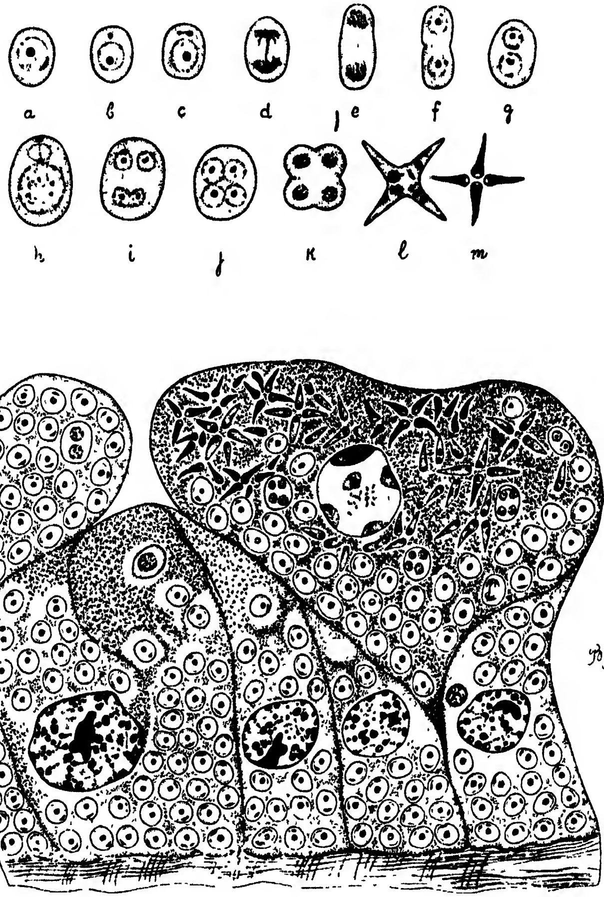


FIG. 315.—*Gurleya francottei*, PARASITIC IN INTESTINAL EPITHELIUM OF LARVÆ OF *Ptychoptera* (× *ca.* 2,000): STAGES OF DEVELOPMENT OF INDIVIDUAL PARASITES AND SECTION OF INTESTINAL EPITHELIUM, SHOWING PARASITES *in situ.* (AFTER LÉGER, L., AND DUBOSCQ, O., 1909.)

a-f. Stages in multiplication by binary fission. *g* Form with two unequal nuclei. *h-m.* Division of nuclei in pansporoblast and formation of four spores.

are enclosed in a thin capsule derived from the superficial layer of the pansporoblast.

Thelohania varians (Léger, 1897).—This is a common parasite of the larvæ of *Simulium reptans* and *S. ornatum,* which are often heavily infected. The cells of the body are seen to be filled with uninucleate

parasites, forms with two nuclei about to divide, and some multinucleate forms which divide into uninucleate forms. Division may take place in such a way that rows of small forms are produced. In addition to the vegetative forms, the cells contain spores in clusters of eight and others more irregularly arranged. The spores vary in length from 4 to 5 microns. Another species, *T. chætogastris*, described by Schröder (1909), is parasitic in an oligochæte worm (Fig. 316).

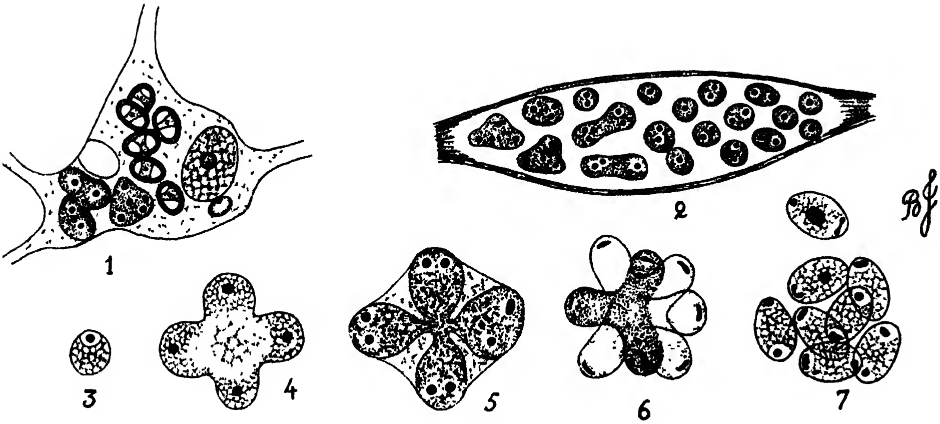


FIG. 316.—*Thelohania chætogastris*, PARASITIC IN THE OLIGOCHÆTE WORM, *Chætogaster diaphanus*. (AFTER SCHRÖDER, 1909.)

1. Connective tissue cell containing three schizonts and spores (× *ca.* 1,500).
2. Muscle cell with reproducing forms (× *ca.* 1,500)
3-7 Stages in formation of eight spores from the single pansporoblast (× *ca.* 2,500).

Genus: Stempellia Léger and Hesse, 1910.

Stempellia mutabilis Léger and Hesse, 1910.—This parasite occurs in the cells of the fat body of the nymph of *Ephemera vulgata*. It resembles very closely a *Thelohania*, except that the pansporoblasts give rise to one, two, four, or eight spores. The latter vary in length from 2 to 6 microns, the largest spore being formed when the pansporoblast gives rise to only one spore and the smallest when it gives rise to eight. The only other member of the genus is *S. magna* (Kudo, 1920), parasitic in larvæ of *Culex pipiens* and *C. territans* in North America.

Genus: Duboscqia Pérez, 1908.

Duboscqia legeri Pérez, 1908.—This form is a parasite of the termite *Termes lucifigus*. It gives rise to white nodules up to 500 microns in diameter in the body cavity. Each nodule shows a peripheral layer of multinucleate cytoplasm, within which are a number of large nuclei up to 60 microns in length. The latter are probably the hypertrophied nuclei

of the infected cells. The central part of the nodule is occupied by pansporoblasts measuring 12 by 7 microns, and groups of sixteen spores enclosed by a capsule. Each group is developed from one pansporoblast. The spores, which are ovoid, measure 5 by 2·5 microns. This genus is evidently closely related to *Thelohania*, with which it may be identical.

Genus: **Plistophora** Gurley, 1893.

The Microsporidiida belonging to this genus are found in fish. They produce small white nodules in the tissues. The pansporoblast gives rise to more than sixteen spores.

Plistophora typicalis Gurley, 1893. — This parasite occurs in the stickleback and other fresh-water fish, in which it gives rise to whitish nodules in the muscles. These are 25 to 35 microns in diameter. Each pansporoblast gives rise to numerous (more than sixteen) ovoid spores, which eventually fill the nodules. Other forms are *P. stegomyiæ* of *Stegomyia fasciata* (*Aëdes argenteus*), and *P. simulii* of larvæ of *Simulium*.

Genus: **Cocconema** Léger and Hesse, 1921.

The Microsporidiida belonging to this genus are characterized by their spherical spores, which resemble cocci (Fig. 318). Léger and Hesse (1921) have described four species from aquatic larvæ or worms.

C. micrococcus occurs in the fat body of the larvæ of *Tanypus setiger*. The spore has a diameter of 1·8 to 2 microns.

C. polyspora occurs in the same host and in the same situation, but the spores are larger, varying in diameter from 2 to 3·2 microns.

C. octospora, the spores of which have a diameter of 2·1 microns, is found in the intestinal epithelium of larvæ of *Tanytarsus* sp.

C. slavinæ with spores 3 microns in diameter, occurs in the intestinal epithelium of the aquatic worm, *Slavina appendiculata*.

Kudo (1924*b*) recognizes two other species—*C. stempelli* and *C. miyairii*.

Genus: **Mrazekia** Léger and Hesse, 1916.

The members of this genus produce cylindrical spores which, in addition to a polar filament, possess a manubrium, which may be regarded as the thickened proximal part of the filament (Fig. 317). The manubrium occupies the central axis of the spore, and the filament is coiled round it. Both the manubrium and filament are extruded. When spore formation takes place, a single uninucleate cell or pansporoblast gives rise to one, four, eight, or sixteen spores.

M. caudata Léger and Hesse, 1916.—This species is parasitic in the lymphocytes of aquatic worms of the genera *Tubifex* and *Limnodrilus*.

The spore, which measures 16 to 18 microns in length and 1·3 to 1·4 in breadth, has the end opposite that from which the filament is extruded drawn out into a pointed process as long as the spore itself. The pansporoblast gives rise to one spore (Fig. 317, 1).

M. brevicauda Léger and Hesse, 1916.—This form occurs in the fat body of larvæ of *Chironomus plumosus.* The spore, which measures 20 to 30 microns by 1·4 to 1·5 microns, has a short pointed process. The pansporoblast gives rise to one spore (Fig. 317, 2).

M. stricta Léger and Hesse, 1916.—This form occurs in the lymphocytes of the aquatic worm, *Lumbriculus variegatus.* The spores, which

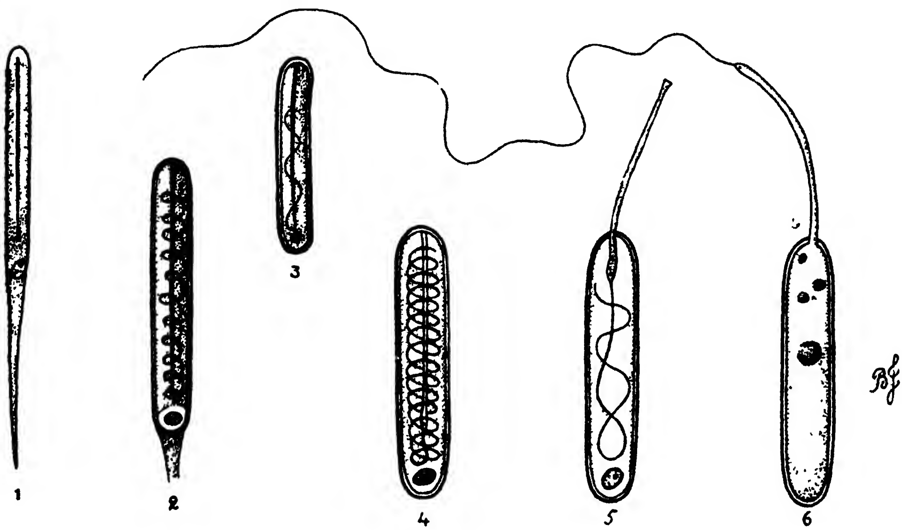


FIG. 317.—SPORES OF MICROSPORIDIIDA OF GENUS *Mrazekia* (× 1,750). (AFTER LÉGER AND HESSE, 1916.)

1. *M. caudata.* 2. *M. brevicaudata.* 3. *M. stricta.*
4. *M. argoisi.* 5. *M. argoisi*: manubrium extruded.
6. *M. argoisi*: complete extrusion of manubrium and polar filament.

measure 13 to 14 microns by 1·8 to 2·0 microns, have no process. Each pansporoblast gives rise to one spore (Fig. 317, 3).

M. argoisi Léger and Hesse, 1916.—This is a parasite of the fat body of the fresh-water crustacean, *Asellus aquaticus.* The spores, which have no pointed process, measure 17 to 23 microns by 3·5 microns. Each pansporoblast forms a single spore (Fig. 317, 4-6).

M. bacilliformis Léger and Hesse, 1922.—This species is a parasite of the fat body of larvæ of *Orthocladius* sp. The spores measure 5 by 0·8 microns, and each pansporoblast gives rise to eight spores.

M. tetraspora Léger and Hesse, 1922.—This form occurs in the fat body of larvæ of *Tanypus* sp. The spores are 6·5 to 8 microns in length

by 0·8 micron in breadth. There is a short hyaline prolongation 1·2 microns long at one end of the spore. The pansporoblast gives rise to four spores.

M. niphargi Poisson, 1924.—This is a parasite of the amphipod, *Niphargus stygius.* The spores measure 8 to 9 by 2 microns. The pansporoblast gives rise to eight or sixteen spores.

M. piscicola Cépède, 1924.—This is the first species of the genus to be described from a vertebrate. It occurs in the pyloric cœca of the whiting, *Gadus merlangus.*

A closely allied, if not identical, genus is *Myxocystis* Mrazek, 1897. These Microsporidiida give rise to white spheres, often ciliated externally, which float about in the body-cavity spaces of aquatic worms (*Limnodrilus*). Mrazek (1897), who first described these forms, later (1910) demonstrated that the white spheres were agglomerations of wandering cells, the cytoplasm of which was infected with uninucleate Microsporidiida, which multiplied by binary fission. Each uninucleate pansporoblast gave rise to a single spore. The spores are ovoid, the narrow end being drawn out into a kind of neck. The spores of *M. mrazeki* Hesse, 1905, parasitic in *Limnodrilus hoffmeisieri*, measure 9 to 10 microns by 1 to 2 microns.

Genus: Octosporea Flu, 1911.

This genus was created by Flu (1911) for a parasite of the intestinal epithelium of the house fly. He thought it was a schizogregarine, but Chatton and Krempf (1911) proved that it belonged to the Microsporidiida. The spores are bacillary and slightly curved. No details of their structure could be made out in the fresh condition, and there was no indication of a manubrium. The pansporoblast gives rise to eight or exceptionally sixteen spores.

Octosporea muscæ domesticæ Flu, 1911.—This parasite was first seen by Flu (1911) in various tissues of the house fly. Chatton and Krempf (1911) saw it in *Drosophila confusa* and *D. plurilineata*, and first realized that it was a microsporidian. The young forms, 3 microns in diameter, occur in the epithelial cells of the intestine. Reproduction takes place by schizogony, forms with as many as thirty-two nuclei occurring. The spores are 5 to 6 microns in length by 1 micron in breadth. The only other species is *O. monospora* Chatton and Krempf, 1911, parasitic in the same species of *Drosophila* and *Homalomyia scalaris.* The spores are 4 to 5 microns in length.

Genus: Toxonema Léger and Hesse, 1922.

Toxonema vibrio Léger and Hesse, 1922.—This parasite is the only member of the genus (Fig. 318). The total length of the spore is 3·5

microns, and it is curved so that the distance between its two ends is 2 microns. Each pansporoblast gives rise to eight spores *T. vibrio* occurs in the fat body of larvæ of species of *Ceratopogon.*

Genus: Spirillonema.

Léger and Hesse (1922*a*) suggested the generic name *Spironema* for those Microsporidiida which have spiral spores resembling spirilla (Fig. 318). As the name *Spironema* was given by Klebs (1892) to a flagellate, the name *Spirillonema* may be used.

Spirillonema octospora (Léger and Hesse, 1922).—This parasite, the only member of the genus, is found in the fat body of larvæ of *Ceratopogon.* The spiral spore is 8 to 8·5 microns long and 1 micron wide. Each pansporoblast gives rise to eight spores (Fig. 318).

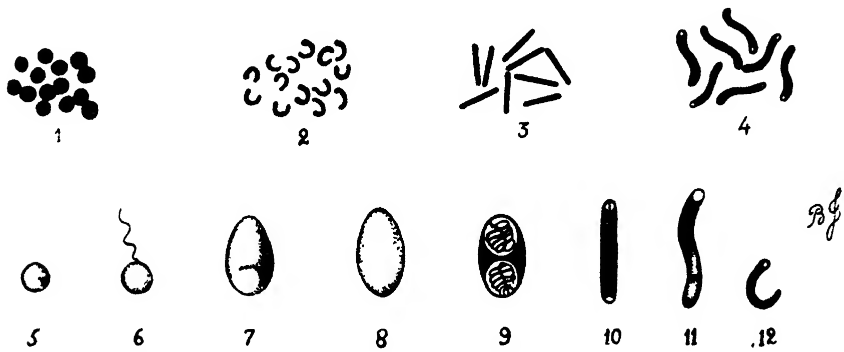


FIG. 318.—SPORES OF MICROSPORIDIIDA. (AFTER LÉGER AND HESSE, 1922.)

1-4. Bacterial types of spore deeply stained as they appear in smears or sections of tissue (× 1,000).
5-12. Types of spore to show some details of structure (× 3,000).
1, 5, 6. *Cocconema* 2, 12. *Toxonema vibrio.*
3. *Mrazekia bacilliformis.* 4, 11. *Spirillonema octospora.*
7. *Glugea.* 8, 9. *Telomyxa*, living and stained. 10. *Mrazekia tetraspora.*

Genus: Telomyxa Léger and Hesse, 1910.

Telomyxa glugeiformis Léger and Hesse, 1910.—This form, which is the only representative of the genus, occurs as a parasite of the cells of the fat body of the larvæ of *Ephemera vulgata* (Fig. 318). The spores are ovoid, and measure 6·5 by 4 microns. There is a polar capsule at each end of the spore. After the multiplicative phase, certain uninucleate cells become pansporoblasts, and each gives rise to groups of eight, sixteen, or more cells, which become transformed into spores. Léger and Hesse regarded this organism, the spores of which have two polar capsules, as a connecting link between the Myxosporidiida and Microsporidiida.

Microsporidiida of Blood-Sucking Arthropoda and Nematoda.

As already remarked, the Microsporidiida are parasites chiefly of Arthropoda, and some of the forms which may be encountered in experimental work will be considered briefly. It is important to remember these organisms when insect flagellates are being studied. They may be confused very readily with the minute leishmania forms of certain flagellates, as pointed out by Chatton (1911*a*) and Shortt (1923), a mistake which undoubtedly has been made on more than one occasion.

MOSQUITOES.—Hesse (1904, 1904*a*) found a parasite in the cells of the fat body of larvæ of *Anopheles maculipennis* in France. The spores of this organism, which Hesse named *Thelohania legeri*, measured 8 by 4 microns. The filament, which was extruded from the spores when placed in iodine water, measured 50 microns in length. The mosquito larvæ seemed to be unaffected by the presence of the parasite. Another species, *T. illinoisensis*, was described by Kudo (1921) from the larvæ of *A. punctipennis* and *A. quadrimaculatus* of North America. It was very similar to Hesse's species, *T. legeri*. The spores, however, appeared to be smaller (4·75 to 6 microns), while the filament was longer (60 to 97 microns). In a later paper Kudo (1924) describes this form in detail, and compares it with *T. legeri* in films from *A. maculipennis* and *A. bifurcatus* larvæ sent to him by Hesse. He comes to the conclusion that *T. illinoisensis*, which occurs in larvæ of *A. crucians*, as well as the mosquitoes mentioned above, is identical with *T. legeri*. It appears to be a parasite specific to larvæ of *Anopheles*, and develops in the cells of the fat body. Reproduction takes place by repeated binary fission. Eventually, forms with four nuclei are produced (Fig. 319). These divide into two sporont mother cells, each with two nuclei. The two nuclei divide, and this is followed by division of the mother cell, so that again stages with two nuclei are produced. The two nuclei then fuse, and at the same time fine chromatin granules appear in the cytoplasm. The cell with a single nucleus is the sporont, which by successive nuclear division, the first of which is mitotic, reaches an eight-nuclear stage. Within it eight sporoblasts are formed, and each of these becomes a spore.

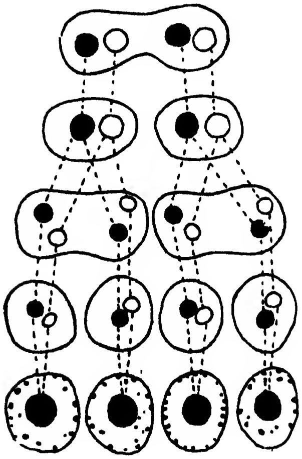


FIG. 319.—DIAGRAM OF NUCLEAR CHANGES IN LATE SCHIZOGONY AND EARLY SPOROGONY OF *Thelohania legeri*. (AFTER KUDO, 1924.)

The two nuclei in the final products of multiplication fuse to form the nucleus of the sporont, which eventually produces the spores.

Kudo (1922*a*, 1924*a*) has given the name *T. opacita* to a parasite of larvæ of *Culex testaceus* (*C. apicalis*) and *C. territans*, also of North America (Fig. 320). The name was suggested by the effect the parasite has on its host, which becomes of an opaque white colour. Its developmental

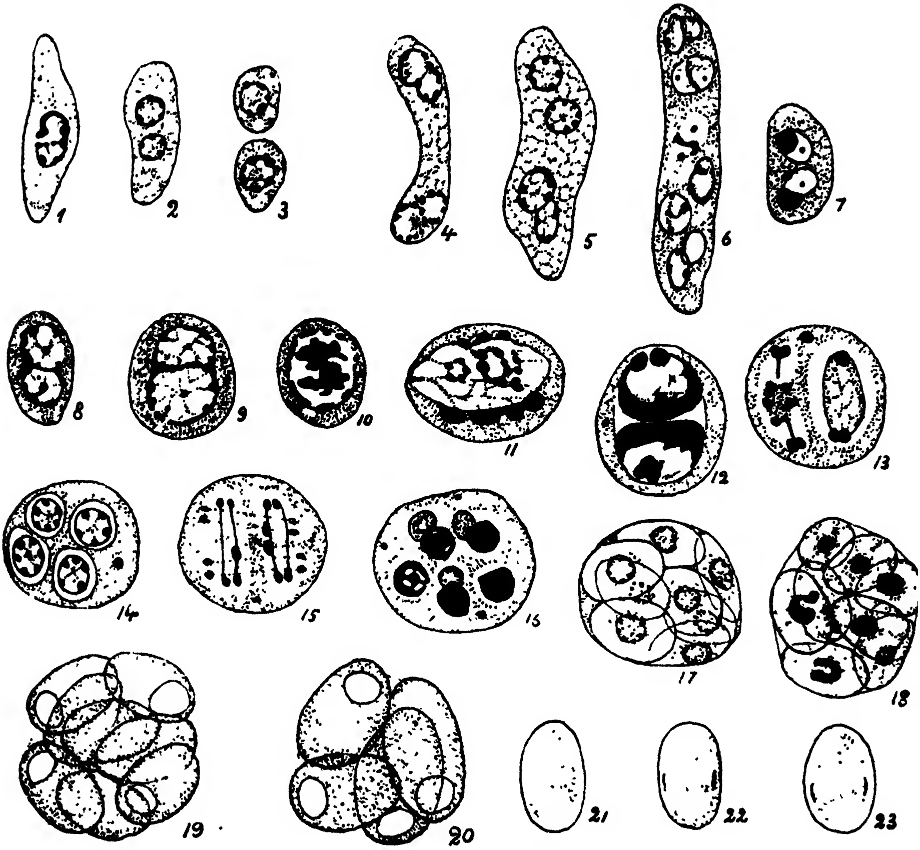


FIG. 320.—DEVELOPMENTAL STAGES OF *Thelohania opacita* (1-20, ×2,300, 21-23, ×2,360). (AFTER KUDO, 1924.)

1-3. Binary fission. 4-6. Multinucleate forms.
7. Final binucleate product of multiplication.
8-10. Union of nuclei to form sporont.
11-16. Division of nuclei to form eight. 17. Formation of eight sporoblasts.
18. Pansporoblast containing eight young spores.
19. Pansporoblast containing eight mature spores.
20. Pansporoblast with four spores. 21-23. Normal-sized spores.

cycle is very similar to that of *T. legeri*. Reproduction is by repeated binary fission. Finally, binucleate forms are produced. These, by fusion of the nuclei, give rise to sporonts (pansporoblasts). The rounded pansporoblast produces, as a rule, eight sporoblasts, which become spores.

These are ovoid in shape, and measure 5·5 to 6 by 3·5 to 4 microns. The polar filament is 110 microns in length. Occasionally, the pansporoblast gives rise to only four sporoblasts, which produce correspondingly larger spores, measuring 8 to 8·5 by 4·5 to 5·5 microns. The polar filament in these cases reaches a length of 200 microns.

The same author (1924*b*) describes as *T. obesa* a parasite of the fat body of an anopheline (*A. quadrimaculatus* ?) larva. The pansporoblast, which gives rise to a group of eight spores, is 9 to 10 microns in diameter. The fixed and stained spore measures 4 to 4·5 by 3 to 3·5 microns.

Another species recorded by this author (1924*b*) is *T. pyriformis* from the fat body of larvæ of *A. crucians* or *A. quadrimaculatus*. The fixed and stained spore measures 3·5 to 4 by 2 to 2·8 microns. In the fresh condition it appears considerably larger, and measures 4·8 to 5·4 by 2·7 to 3 microns.

Kudo (1920, 1921) described as *T. magna* a microsporidian of the larvæ of *C. pipiens* and *C. territans* in North America. Later (1924*b*) he transferred it to the genus *Stempellia* (Fig. 313). It occurs in the cells of the adipose tissue, and the larvæ were heavily infected. The parasite multiplies by binary fission or by schizogony. Finally, a division of a parasite into four cells, which remain connected together, takes place. A further division of each of these may occur. The resulting cells are sporoblasts, which develop into spores. The spore measures 12·5 to 16·5 by 4 to 4·6 microns. The extruded filament may reach a length of 350 to 400 microns.

Two other species of *Thelohania* are recorded by Kudo (1924*b*) from *Culex leprincei* of North America. One of these is named *T. rotunda*. The spore is broadly ovoid or sub-spherical, and when fixed measures 2·5 to 3 by 2·3 to 2·7 microns. The other, *T. minuta*, has an ovoid spore measuring when fresh 3·5 to 3·7 by 2·4 to 2·7 microns, and when fixed 2·5 to 3·3 by 1·5 to 2 microns. Both occur in the adipose tissue of the larvæ, while *T. minuta* has been found in the pupæ also.

The writer has seen a *Thelohania* which was discovered by MacGregor in larvæ of *Aëdes* (*Ochlerotatus*) *nemorosus* in England. The fresh spores measured 6 to 7 by 4 to 4·5 microns. The parasite occurred chiefly in the fat body, and appeared to be specific for the larvæ of this particular mosquito, as the larvæ of other mosquitoes, including other species of the same genus, in the pond at the same time were not infected. Attempts to infect larvæ of *O. nemorosus* from another locality and larvæ hatched in the laboratory failed, though enormous numbers of the spores were ingested. No infection of the body cavity took place. In the pond, in which at one time early in March at least 50 per cent. of the larvæ of this species were infected, the infection gradually died out during the course of one month, though

there appeared to be every chance of its survival in the larvæ of *O. nemorosus*, which were constantly present. It seems evident that infection depends on certain conditions not at present known.

Another form described by Kudo (1924*b*) is *Nosema anophelis*, a parasite of larvæ and adults of *A. quadrimaculatus*. In the larvæ it occurred in the gastric pouch, and in the adults in the epithelial cells of the anterior part of the mid-gut and in the neighbouring fat body. The young forms, which reproduce by binary fission, are 1·5 microns in diameter. Each pansporoblast produces a single spore, which measures from 4·7 to 5·8 microns in length by 2·3 microns in breadth. The filament is 50 to 60 microns in length.

Marchoux, Salimbeni, and Simond (1903) described as *N. stegomyiæ* a parasite of the larvæ and adults of *Aëdes argenteus* (*Stegomyia fasciata*) in Brazil. It occurs in the intestine, body cavity, and tissues of the posterior part of the body, including the ovaries. It is supposed that two kinds of spore are produced, the one colourless, the other brown. The colourless spore gives rise to multinucleate plasmodia up to 40 microns in diameter, and the brown spore to long filaments. There is considerable doubt regarding the description of the parasite, the microsporidian nature of which has not actually been demonstrated. Chatton (1911*a*) placed the parasite in the genus *Plistophora*.

Bresslau and Buschkiel (1919) recorded as *Thelohania* sp. a parasite of larvæ of *Theobaldia annulata* in Germany. Nöller (1920*b*) mentions the occurrence of a parasite which he supposes to be *Thelohania legeri* in larvæ of *Aëdes nemorosus* in Germany. As *T. legeri*, according to Kudo (1924), is specific for *Anopheles*, it is probably some other species. Nöller also mentions *Nosema* sp. as occurring in *Aëdes nemorosus* and *A. cantans*. Bresslau and Buschkiel (1919) gave the name *Nosema culicis* to a parasite of larvæ of *Culex pipiens*. The spores measured 4·5 to 5·5 by 1·8 to 2·4 microns. What are possibly spores of Microsporidiida were seen by Pfeiffer (1895) in larvæ of *Culex* sp. in Germany, by Grassi (1900) in larvæ and adults of *Anopheles* sp. in Italy, and by Ross (1906) in adults of *C. fatigans* and *Aëdes* sp. in India. Kudo (1921), who has reviewed the literature dealing with Microsporidiida of mosquitoes, doubts if these are, in most cases at least, true Microsporidiida.

SIMULIUM.—The larvæ of various species of *Simulium* are very liable to infection with Microsporidiida. Heavy infections occur, so that the larvæ often appear swollen and white in colour, while in some cases actual nodular tumours are produced. The first form noted was one which occurred in *S. ornatum*, and was named *Glugea varians* by Léger, L. (1897). The parasite was studied by Debaisieux (1919*a*), who found it also in *S. reptans*. He transferred it to the genus *Thelohania*. It occurs

in the body cavity and adipose tissue, and produces spores measuring 6·5 to 8 by 4·5 to 5·5 microns. Strickland (1913) in America named three species which he placed in the genus *Glugea*. They were transferred to the genus *Thelohania* by Debaisieux and Gastaldi (1919), who found them in Belgium. *T. bracteata* and *T. fibrata* were found in *S. venustum* and *S. ochraceum* in South America by Lutz and Splendore (1904, 1908), in *S. bracteatum* and *S. hirtipes* by Strickland (1913) in North America, and in *S. maculata* by Debaisieux and Gastaldi (1919) in Belgium. The parasites occur in the fat body of the larvæ. The spores of *T. bracteata* measure 3 to 4 by 2·5 to 3 microns, and those of *T. fibrata* on an average 7 by 3·5 microns. Another species, *T. multispora*, seen by Strickland (1913) in *S. vittatum* and *S. bracteatum*, and by Debaisieux and Gastaldi (1919) in *S. maculata*, also occurs in the fat body, but produces spores of an intermediate size.

Lutz and Splendore (1904) included in their species *Nosema simulii*, which embraced the forms noted above, a parasite which Debaisieux and Gastaldi (1919) placed in the genus *Plistophora*. It was seen in *S. venustum* and *S. ochraceum* by Lutz and Splendore, and in *S. maculata* by Debaisieux and Gastaldi. It produces regular rounded tumours in the tissues of the larvæ. The spores vary in size from 4·5 to 8·5 by 3·5 to 5·5 microns.

FLEAS.—These arthropods are also liable to infection with Microsporidiida. Nöller (1912) found a form which he named *Nosema pulicis* in the salivary glands, Malpighian tubes, and fat body of the dog flea (*Ctenocephalus canis*) in Germany. The oval spores measured 2·5 to 5 microns in length by 1·5 to 2 microns in breadth. The polar filament was 65 to 85 microns long. Another form was described by Korke (1916) from the dog flea in India. He suggested the name *N. pulicis*, but as the spores are smaller than those of *N. pulicis*, Kudo (1924*b*) has given it the name *N. ctenocephali*. Shortt (1923), in a study of *Leptomonas ctenocephali* of the dog flea, has drawn attention to the care which must be exercised in distinguishing the spores of *N. ctenocephali* from leishmania forms of the flagellate.

BED BUGS.—Certain small ovoid bodies which Adie (1922, 1922*a*) found in the salivary glands and other tissues of bed bugs in India, and which were regarded as stages of *Leishmania donovani*, are, according to Christophers (1922), Microsporidiida, for which he proposes the name *Nosema adiei* (see p. 420). The parasite has been described by Shortt and Swaminath (1924*a*), who have also met with it in bugs in India. The intestine is most commonly infected. The spores are ovoid or elliptical bodies measuring 3 by 1·7 microns. Minute small amœbulæ 1·6 microns in diameter occur, as also larger forms measuring 3·2 by 2·7 microns.

These free forms in dried films stained by Romanowsky stain have blue protoplasm and one or two red chromatin areas. When there are two of unequal size, some resemblance to *Leishmania donovani* may result, but the smaller of the two red areas is never rod-shaped.

NEMATODES.—Lutz and Splendore (1908) gave the name *Nosema mystacis* to a parasite of the intestinal cells and reproductive organs of *Ascaris*

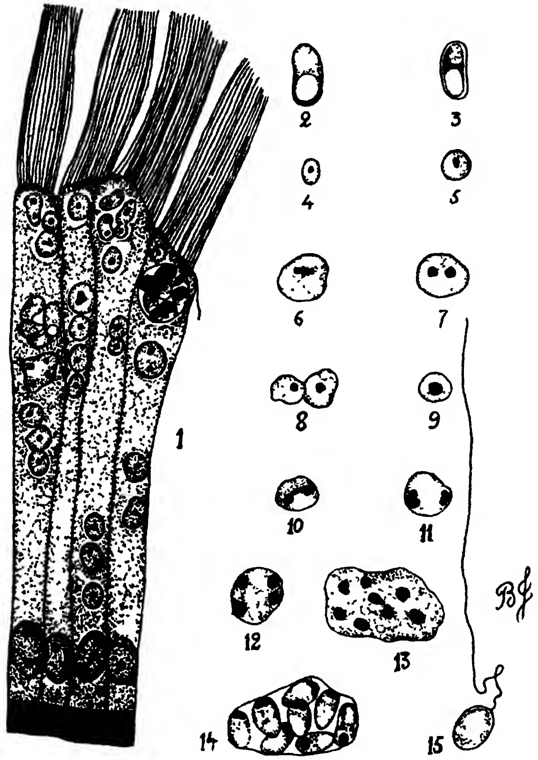


FIG. 321.—*Thelohania reniformis*, PARASITIC IN INTESTINAL EPITHELIUM OF *Protospirura muris* (1, ×1,560; 2-15, ×2,200). (AFTER KUDO, 1923.)

1. Four cells of intestinal epithelium showing various stages of parasite.
2. Fresh spore. 3. Stained spore.
4-8. Growth and multiplication by binary fission.
9-14. Growth of pansporoblast and formation of eight spores.
15. Spore with extruded filament.

mystax of Brazilian cats. The bodies seen by Bischoff (1855) and Keferstein (1861) in the same helminth were possibly spores of this species. Kudo and Hetherington (1922) have described as *Thelohania reniformis* a parasite which they found in the lining cells of the intestine of *Protospirura muris*, a common helminth of the stomach of mice (Fig. 321). The

spores are reniform, and measure 3 to 4 by 1·5 to 1·8 microns. The polar filament is 45 to 55 microns in length. The pansporoblast gives rise to eight sporoblasts, which become eight spores.

Supposed Microsporidiida in Rabies and Encephalitis of Rabbits and Mice.

Wright and Craighead (1922) observed a form of paralysis in young rabbits, and found that it was due to an organism which they thought might be a Protozoon. It was found in most of the tissues of the body, but was specially noticed in the kidneys and urine and in nerve cells of the spinal cord, which were quickly destroyed

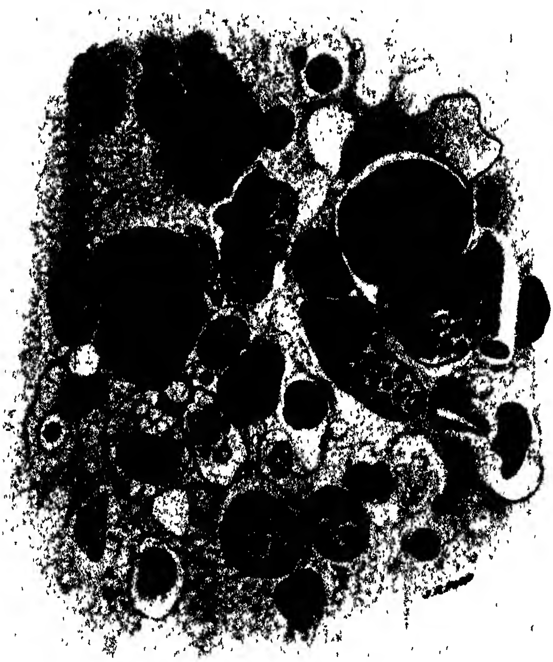


FIG. 322.—*Encephalitozoon cuniculi* IN SECTION OF BRAIN OF RABBIT (×950). (AFTER DA FANO, 1924, FROM *Journ. Path. and Bact.*)

Parasites in cysts which may be merely vacuoles in macrophages.

by its presence. The same disease of rabbits had been observed by Bull (1917), Oliver (1922), and Twort (1922). They described the changes in the nervous system without associating them with any particular organism. In attempts to reproduce human diseases, encephalitis lethargica and herpes, in rabbits, the naturally occurring disease of rabbits and the associated organism have given rise to some confusion. Doerr and Zdansky (1923) described the lesions in the brains of rabbits inoculated with the virus of encephalitis lethargica, and discovered that similar lesions occurred in uninoculated animals. They saw in the brain certain small bodies which they thought were probably parasites responsible for a disease of rabbits which was being

confused with encephalitis lethargica. Later in the year Levaditi, Nicolau and Schoen (1923) also saw the organism, and recognized it as the cause of an encephalitis of rabbits which had no connection with the human disease. They gave it the name *Encephalitozoon cuniculi*, and expressed the opinion that it was a microspordian. Doerr and Zdansky (1923*a*, *b*) then gave a clear description of the organism. They noted that it occurred in the form of spores, which were either distributed through the tissues of the brain or collected together in cysts. Levaditi, Nicolau and Schoen (1924*a*) have given a complete review of the subject and described their own results. They were able to inoculate the organism to rabbits, rats, mice, and dogs. In rabbits it was found only in the brain and kidneys, though Wright and Craighead (1922) had observed it also in the spleen, liver, and myocardium, as also in the urine, which suggested to them a possible source of infection. Levaditi and his co-workers have actually demonstrated the infectivity of the urine.

FIG. 323.—*Encephalitozoon cuniculi* IN ATROPHYING NERVE CELL AND SCATTERED THROUGH THE BRAIN SUBSTANCE (× 1,200). (AFTER DA FANO, 1924.)

During the course of their experiments they (1924) discovered that mice were liable to a similar infection. Cowdry and Nicholson (1924) have also observed an organism in mice, which appears to be morphologically identical with that in rabbits, and gives rise to similar lesions in the central nervous system. The lesions consist of meningitis of the cortex and septa of the brain, perivascular infiltration of the blood-vessels, and nodules composed of masses of cells which may be necrotic centrally. Marked changes occur in the kidneys, especially in the heavily infected epithelium of the tubules, and in the liver and spleen.

In the infected organs the parasites are either scattered through the tissues or enclosed in masses in what are called cysts (Figs. 322, 323). It appears more probable that these are the remains of endothelial or other cells, for often a large flattened nucleus can be seen on the cyst wall. In fact, Wright and Craighead describe the infected cells as being reduced to membranes containing the organisms. By the rupture of the enclosing membrane the spores are scattered through the tissues.

The individual parasite is a small ovoid body about 2·5 microns in length by 0·5 to 1 micron in breadth. At the end there are one or two chromatin-like granules. In many respects it resembles a small yeast, but reproduction by budding has not been observed. Levaditi and his co-workers believe that they have demonstrated small cytoplasmic bodies (pansporoblasts), which give rise to the spores, and they conclude that the parasite is a microsporidian, in spite of the fact that these parasites have never been found in warm-blooded vertebrates. They have not demonstrated the presence of a polar capsule and filament, while their account of the development of the spore requires confirmation. It seems premature to conclude that the organism is even a Protozoon. The writer in 1909 saw what was evidently the same organism in sections of the brain and liver of a rabbit, but was unable to arrive at any conclusion regarding its nature. Da Fano (1924) has given a good description of the organism and the lesions it produces in the brain of rabbits in England, and Smith and Florence (1925) its appearance in the kidneys. Goodpasture (1924) has seen it in the lungs.

Levaditi, Nicolau and Schoen (1924*b*) suggest that the virus of rabies is probably a microsporidian which enters the body in some invisible stage, and produces eventually the Negri body. Manouelian and Viala (1924) go even further, and claim to have demonstrated in the cells of the brain and salivary gland of dogs organisms which are indistinguishable morphologically from those in the disease of rabbits described above. They name the organism *Encephalitozoon rabiei.* Levaditi, Nicolau and Schoen (1924*c*), in a later paper, confirm the observations of Manouelian and Viala, and claim that the Negri body is the cyst stage of the parasite. Ignoring the specific name *rabiei*, they place it in the genus *Glugea* as *G. lyssæ.* Here, again, there is no evidence that the organism is a microsporidian, as indeed Manouelian and Viala suspect.

The presence of the parasite in mice, whatever its true nature may be, introduces another fallacy into experiments which involve the discovery of *Leishmania* in the organs of animals inoculated with insect flagellates (see p. 395). The figures of *Encephalitozoon cuniculi* and the similar parasite of mice show how easy it would be for such parasites, when seen in smears stained with Romanovsky stain, to be mistaken for *Leishmania.*

C. *Order:* ACTINOMYXIDIIDA.

The parasites included in this order (=Actinomyxidia Štolc, 1899), which were first seen and named by Štolc (1899), occur in aquatic worms. The spores are complicated structures consisting of a capsule composed of three valves, each of which may be drawn out into a long spine which may be bifurcated, so that there is a definite triradiate arrangement (Fig. 324). Three polar capsules are present, and the mature spore contains a variable number of amœboid infective agents, often referred to as sporozoites. The Actinomyxidiida have been studied by Štolc (1899), Léger, L. (1904*a*), Caullery and Mesnil (1905*a*), Ikeda (1912), and Mackinnon and Adam (1924), to whose researches most of what is known of these parasites is due. The development of the spore is a complicated process which resembles that of the spores of Myxosporidiida. It has been traced in certain species by Caullery and Mesnil (1905*a*), Ikeda (1912), and Mackinnon and Adam (1924). There are, according to Ikeda, the following five genera: *Tetracti-*

nomyxon, *Hexactinomyxon*, *Triactinomyxon*, *Synactinomyxon*, and *Sphæractinomyxon*, which differ from one another in the character of the spores and other details.

According to the observations of Mackinnon and Adam (1924) on *Triactinomyxon legeri* in *Tubifex tubifex*, the life-history is as follows (Fig. 325): The pansporocyst, a spherical cyst of about 60 microns in diameter, contains eight closely packed spores, the three tails of which are

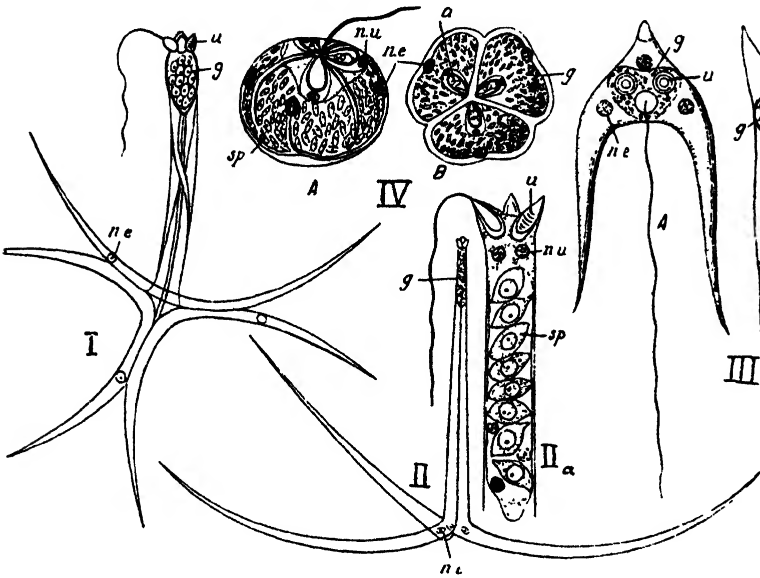


FIG. 324.—SPORES OF VARIOUS ACTINOMYXIDIIDA. (FROM CAULLERY AND MESNIL, 1905, AFTER STOLC, LÉGER, AND CAULLERY AND MESNIL.)

I. *Hexactinomyxon psammorocystis* (× 450). II. *Triactinomyxon ignotum* (× 250). IIa. Terminal portion of spore of *T. ignotum*, showing eight "sporozoites" and three polar capsules (× 900). III. *Synactinomyxon tubificis*. *A*, Surface view of spore with three polar capsules; *B*, side view of spore (× 900). IV. *Sphæractinomyxon stolci*. *A*, Side view of spore; *B*, end view, showing three polar capsules (× 900).

g, Germinal mass; *sp.*, "sporozoite"; *n.e*, nucleus of envelope cell; *n.u*, nucleus of polar capsule cell; *u*, polar capsule.

folded within the cyst membrane. By rupture of the cyst the spores are liberated when the tails become extended, and the characteristic triradiate arrangement is seen. The individual spore varies in size. Its length up to the point where the three rays originate varies between 90 and 140 microns, while its breadth varies from 11 to 16 microns. The rays also vary in length, but on an average this is 150 microns, while the breadth is 8 to 14 microns. At the end of the spore are three polar capsules, from which filaments can be extruded, while adjacent to them is a mass

of cytoplasm (spongioplasm), in which are embedded, in three columns of eight, twenty-four sporozoites. In addition, the spongioplasm contains three nuclei. The spongioplasm, either intact or segmented into several

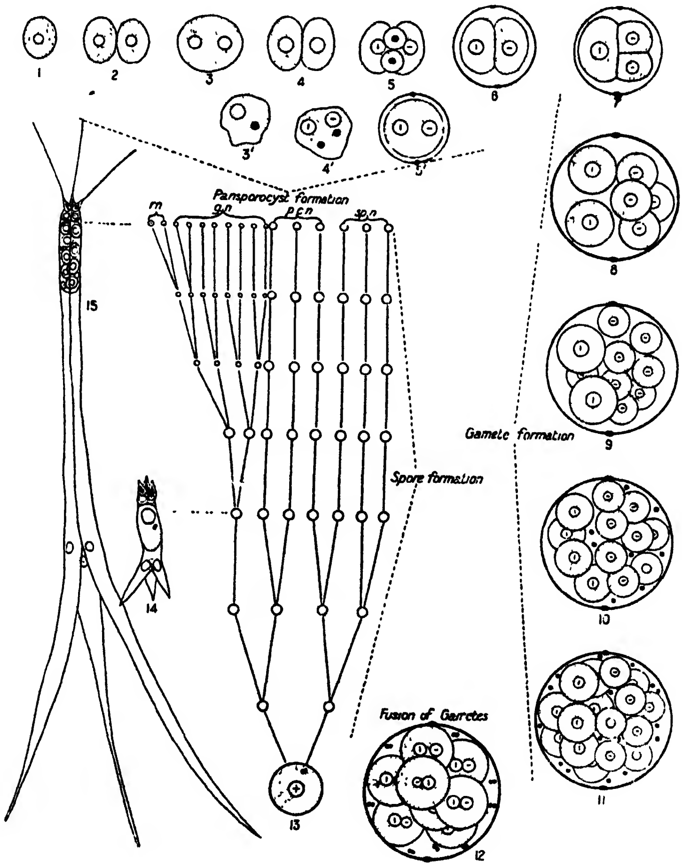


FIG. 325.—DIAGRAM OF LIFE-HISTORY OF *Triactinomyxon ignotum*. (AFTER MACKINNON AND ADAM, 1924, FROM THE *Quart. Journ. Mic. Sci.*)

1. Sporozoite.
2-6 Formation of pansporocyst (3′ to 5′ stages in *T. legeri* given as alternative).
7-11. Formation of gametes and their reduction bodies (black dots).
12. Union of gametes.
13. Zygote and subsequent nuclear divisions (*r.n.*, residual nuclei; *g.n.*, nuclei of sporoplasmic mass; *p.c.n.*, nuclei of polar capsule cells; *sp.n.*, nuclei of sporal envelope).
14. Young spore. 15. Ripe spore.

masses, moves from the region of the polar capsules towards the point of origin of the rays, where a pore is probably present. Meanwhile, the twenty-four sporozoites unite in pairs, giving rise to twelve binucleate amœboid bodies. The nuclei of these, though they may come into contact

with one another, do not fuse. These binucleate amœboid bodies, which are about 11 microns in diameter, escape into the intestine of the worm and proceed to develop, so that auto-infection has to be recognized. The two nuclei of each amœboid body divide to form a total of four nuclei, two of which migrate to the periphery and, together with some of the cytoplasm, form the capsule which develops, while the two remaining nuclei increase in size. The cytoplasm within the capsule, which increases in size, divides to form two cells, while by a further division four cells are produced, two of which are larger than the other two. By further divisions, which proceed somewhat irregularly, eventually eight small cells and eight large cells are formed. These are gametes, and it appears that certain reduction bodies are discharged from their nuclei. The gametes unite in pairs (anisogamy), forming eight zygotes within the cyst. Each zygote, which, in addition to the nucleus, contains a granule of what appears to be residual chromatin, develops into a three-rayed spore. By a series of nuclear divisions, two, four, six, and then seven nuclei are formed. Three of these migrate to one end of the cytoplasmic mass, where they become the capsulogenous cells from which the polar capsules are formed, three of them pass to the opposite pole and form the cells which give rise to the three-rayed spore envelope, while the remaining nucleus divides many times till twenty-seven nuclei are formed. Of these nuclei, twenty-four are nuclei of sporozoites and three the residual nuclei of the spongioplasm, in the substance of which the twenty-four sporozoites are eventually developed.

In this development it will be seen that the union of the sporozoites is not a syngamic process, as the nuclei remain distinct. When the nuclei divide to form four nuclei, two of the nuclei, together with some of the cytoplasm, form the cyst wall, which encloses the rest of the cytoplasm containing two nuclei. Eventually sixteen gametes are produced from these two cells, and it seems probable that eight of these, which are macrogametes, are derived from one sporozoite, while the eight microgametes are formed from the other. Finally, there is a spherical cyst containing eight zygotes. When each zygote has completed its development, the spherical cyst contains eight closely packed spores. The worm *Tubifex tubifex* appears to harbour at least four species of *Triactinomyxon*. *T. ignotum* Štolc, 1899, has eight sporozoites in each spore; *T. legeri* Mackinnon and Adam, 1924, has twenty-four; *T.* sp. Léger, 1904, has thirty-two; and *T. mrazeki* Mackinnon and Adam, 1924, has more than fifty. The cycles of development of these species resemble one another very closely, and it appears probable that, apart from the difference in shape of the spores, the members of the other known genera of Actinomyxidiida develop in a similar manner. Granata (1925), who

recognizes a sixth genus, *Neoactinomyxon*, has recently published a memoir on the morphology, development, and systematics of the group.

PARASITES OF UNDETERMINED POSITION.

There are a number of organisms which are usually grouped with the Cnidosporidia, though they do not show any of their main characters. It is doubtful if some of them are Protozoa at all. The chief of these are the Sarcosporidia, which are parasitic in the muscle fibres of vertebrates, and have the form of elongate chambered bodies filled with sickle-shaped spores; the closely related *Globidium* (cysts of Gilruth), which give rise to nodules in the mucosa of the stomach and intestine of ruminants and other animals; the Haplosporidia, which occur chiefly in invertebrates in the form of uninucleate or multinucleate cytoplasmic bodies and resistant spores; and the Serumsporidia, which are found in the body-cavity fluids of aquatic crustacea and larvæ as small round cells which multiply by binary fission or schizogony. The Rhinosporidia, which produce nasal polypi, have been usually classed with the Haplosporidia, but Ashworth (1923) has shown conclusively that they are fungi, and not Protozoa, and this appears to be true of *Globidium* and possibly the Sarcosporidia.

Sarcosporidia Bütschli, 1882.

The parasites included under this heading are regarded as belonging to the genus *Sarcocystis* Ray Lankester, 1882, and are usually classed with the Cnidosporidia; but, as noted above (p. 717), there are no adequate grounds for this. They are often grouped as a separate order, Sarcosporidia Bütschli, 1882. They are found chiefly as parasites of the striated muscle fibres, and less frequently of the unstriated fibres of mammals; but a few forms have been recorded from birds and reptiles.

They have been found occasionally in man, and are very common in sheep, cattle, and horses. A form which occurs in mice and rats has been studied more than others on account of the fact that experimental infections can be produced by feeding the animals on infected tissues.

Morphology.—In whatever hosts they occur, the parasites are very uniform in appearance, though the forms described vary considerably in size. Since the discovery by Miescher (1843) of a form in muscle fibres of mice, they have frequently been known as "Miescher's tubes," and the spores as "Rainey's corpuscles." They are sometimes so small that they can only be detected with the microscope. At other times they are seen as tiny white streaks in the muscle fibres, while they may be as much as 5 centimetres in length. When seen entire after removal from the tissues,

each parasite has a whitish appearance, is cylindrical with somewhat pointed ends, and has a slightly lobulated surface (Fig. 326). Each is normally embedded in a muscle fibre, but this ultimately degenerates, so that the parasite is left in the connective tissue. There is an enclosing membrane which, in the larger forms, shows a radial striation. It is not quite clear whether this membrane is formed by the parasite or by the tissues. Within the membrane a thin multinucleate layer of cytoplasm is sometimes supposed to be present, and this layer also contains vacuoles in which uninucleate cells occur. In most cases it is impossible to detect such a multinucleate cytoplasm, and all that can be seen is a layer of uninucleate cells lying in spaces in a homogeneous material which may, however, be cytoplasm. This material gives rise to a series of septa, which divide the bulk of the parasite into a number of chambers (Fig. 327). Those near the membrane contain single spherical cells, and are smaller than the ones nearer the centre of the parasite. The more deeply situated chambers are larger and contain a number of round cells, while those that are fully developed contain a variable number of characteristic sickle or crescent-shaped bodies usually called spores. These are covered by a delicate membrane, which cannot be compared with the resistant covering of the spores of Microsporidiida. In old parasites, the central part usually consists of a space filled with free spores in various stages of degeneration and granular débris. The central portion is that which was first formed, and it ultimately degenerates, while the parasite still increases in size and produces new septa and spores peripherally.

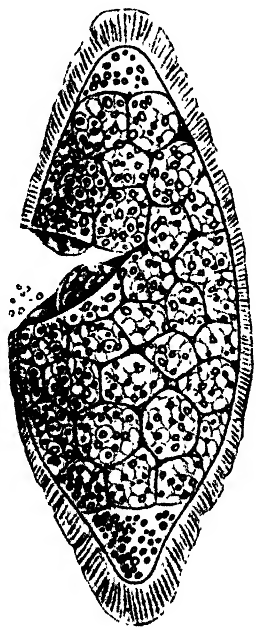


FIG. 326.—SARCOCYSTIS OF THE PIG. (AFTER MANZ, 1867.)

A single cyst from a muscle fibre, showing the striated capsule and a rupture through which can be seen the groups of spores in the chambers.

The spores are presumably produced by multiplication of the peripheral cells which reproduce by binary fission, while the space in which the cell lies dilates to form a chamber. When a number of such cells is present, they become transformed into the spores. The latter, which measure from 10 to 15 microns in length, may be readily stained in dried films by Romanowsky stains, but the true structure can only be made out in properly fixed smears. Each is crescentic in shape, and has one extremity rounded and the other pointed (Fig. 329). Towards the rounded

end is an elongate nucleus consisting of a membrane and central karyosome. The cytoplasm of the spore towards the pointed end is clear and hyaline, while the rest contains a number of granules of a material which stains deeply. The clear portion was supposed to be of the nature of a polar capsule, chiefly as a result of the statements of Van Eecke (1892), who claimed to have produced an extrusion of filaments as occurs in the spores of Microsporidiida. This observation has never been confirmed, and it seems evident that the clear part of the spore contains nothing but hyaline cytoplasm, and in no way corresponds with the polar capsules of the Microsporidiida. A fully-grown parasite will contain many thousands of spores, which escape when rupture takes place.

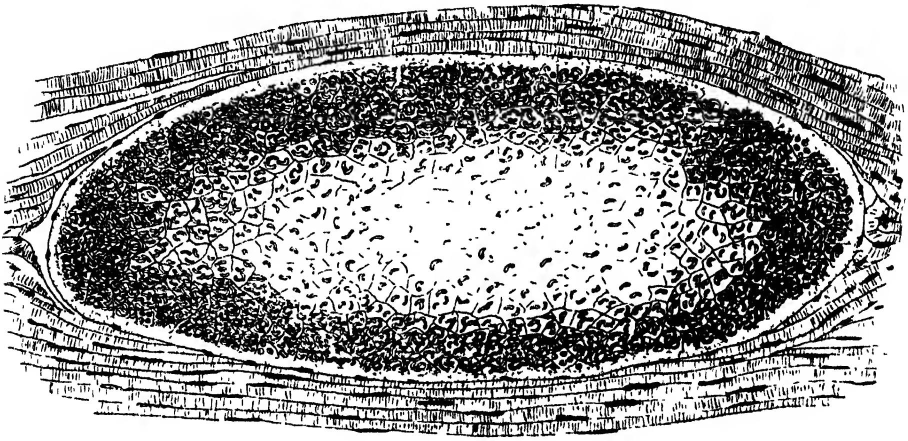


Fig. 327.—Diagrammatic Representation of Longitudinal Section of a Sarcocyst in the Muscles of the Ox (× *ca.* 500). (Original.)

Life-History.—The development of the parasite has been studied chiefly in experimentally infected mice. Erdmann (1910, 1910*a*, 1914) states that in the intestine of the mouse the spore membrane ruptures and liberates a small amœboid body which enters the intestinal cells. There follows a period of multiplication. The parasites persist in the gut wall for a few days only. They then disappear, and are only detected about forty days later in the muscle fibres. According to Negri (1910), who studied experimental infections in rats, the youngest forms seen in muscle fibres have a length of 25 microns, and are cytoplasmic bodies containing about twelve nuclei (Fig. 328). Segmentation of this multinucleate plasmodium then occurs, so that a number of cells are enclosed in a membranous sheath. This development occupies from forty-eight to sixty days. The sheath may rupture, and the cells which are liberated infect

other muscle fibres, so that intense infections of all the muscles may occur. On the other hand, the parasite may increase steadily in size, so that after seventy days it may be half a centimetre in length and contain numerous cells which are apparently formed by division of those originally produced. Towards the centre of the parasite some of the cells are transformed into the sickle-shaped spores. With increase in size larger numbers of spores are formed, and finally the characteristic structure, as described above, is reached.

Alexeieff (1913*a*), in a study of the parasite of sheep, came to the conclusion that the enclosing envelope consisted of three zones, the inner of which is continued as the septa, which enclose the uninucleated cells and spores (Fig. 329). He believes that the envelope, together with the septa, are in reality derived from the host cell, and do not belong to the parasite, which is represented by the round uninucleate cells which multiply by division, and the spores into which they ultimately become transformed. According to this view, each sarcocyst is not a single parasite which is producing spores, but a large number of uninucleate parasites enclosed by an envelope and septa derived from the host. Chatton and Avel (1923), from a study of *S. platydactyli* of the gecko, come to the conclusion that the enveloping membrane belongs to the parasite, and is not developed from the tissues of the host.

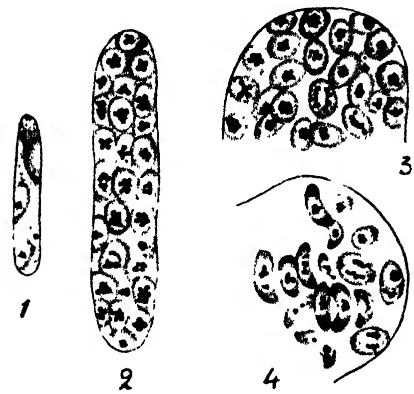


FIG. 328.—*Sarcocystis muris* IN MUSCLES OF MICE. (AFTER NEGRI, 1910.)

1. Form 25 microns in length fifty days after feeding.
2. Form 52 microns in length fifty days after feeding.
3. Section of portion of a parasite fifty days after feeding.
4. Section of portion of a parasite sixty-eight days after feeding.

Negri (1908) believes that he has demonstrated multiplication of the spores themselves by binary fission, not only in the case of *S. muris*, but also *S. bertrami* of the horse, while Teichmann (1911) claims to have made a similar observation in the case of *S. tenella* of sheep.

The parasites in old infections may be as much as 5 centimetres in length. It is quite evident that the dimensions cannot be employed as a means of distinguishing species, so that there is very little evidence that the numerous species of *Sarcocystis* which have been described are valid.

Theobald Smith (1901, 1905) was the first observer to demonstrate that mice could be infected by feeding them with spores. Negri (1910)

and Darling (1910*a*) showed that guinea-pigs could be infected with the parasite of rats, and Darling points out that the forms in the guinea-pig are morphologically identical with those described by him from man. Erdmann

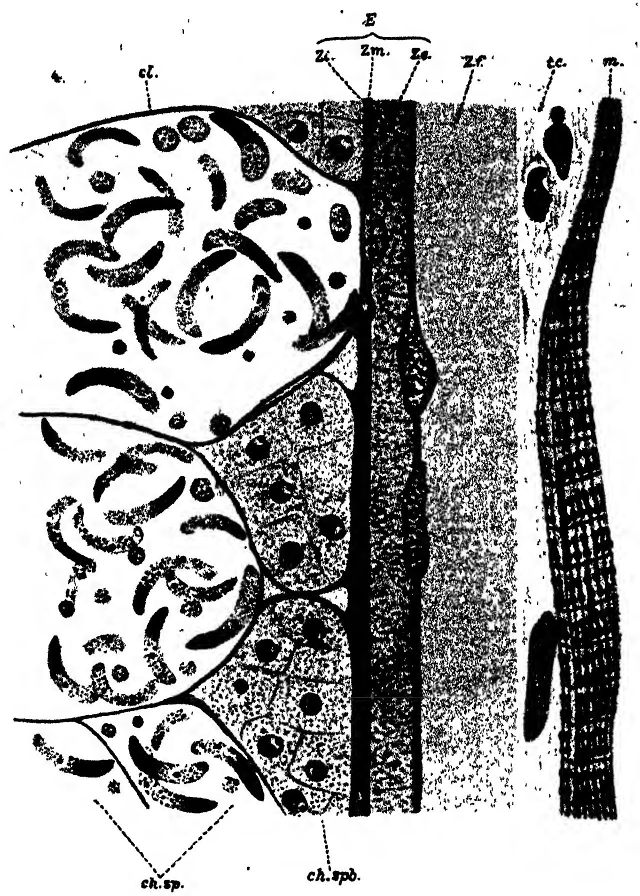


FIG. 329.—*Sarcocystis tenella* OF SHEEP: DIAGRAMMATIC REPRESENTATION OF SMALL PORTION OF CYST (× *ca.* 1,000). (AFTER ALEXEIEFF, 1913.)

E, Envelope of cyst consisting of three zones—external (*Ze.*), median (*Zm.*), and internal (*Zi.*). The latter is continued as the septa, which divide the cyst into chambers. Outside the envelope of the cyst is a fibrous zone (*Zf.*), a connective tissue layer (*tc.*), and a muscular layer (*m.*). The chambers near the envelope contain the sporoblasts (*ch.spb.*), which become transformed into spores (*ch.sp.*). According to Alexeieff, the envelope and the septa are developed from the tissues of the host, the parasites being the cells which become sporoblasts and spores.

(1910*a*) succeeded in infecting mice with the parasite of sheep, while Darling infected guinea-pigs with the form found by him in the opossum. It seems clear that these parasites are not specific to any particular host.

Crawley (1916) gives an account of the early stages of development of the spore in the intestine of mice. He supposes that the spores first differentiate in the lumen of the gut into male and female forms. These enter the epithelial cells and undergo further changes. The nucleus of the male enlarges and its cytoplasm disappears entirely. The nucleus is then supposed to produce microgametes, as in coccidia. The female, however, retains its cytoplasm, and is eventually fertilized by a microgamete. Crawley supposes that the zygote so formed proceeds to multiply in the cell, as Erdmann has described. The description given by Crawley seems very unconvincing, and some of his figures might equally well represent degenerating parasites, while others might conceivably be stages in the development of *Eimeria falciformis*, which is a common intestinal parasite of mice. According to Marullaz (1920), in mice which have been fed on infected material the spores can be found in the intestinal cells within two hours. Soon after this they become round and the nucleus divides by mitosis. Finally, division into two takes place. The daughter forms may escape into the intestine again and infect other cells. Multiplication by binary fission goes on for about ten days. Meanwhile, parasites have been entering the lymphatic spaces of the villi, and thence make their way to the liver and spleen, where the author found them on the eleventh day after feeding. These forms measure 3 by 4 microns, and have a single nucleus. From the forty-fourth to the fifty-fifth day similar forms were found in the muscle fibres. In addition, certain parasites in the muscles had two nuclei, and in one case there appeared to be a division into eight cells. The author regards the last named as an early stage in the development of a typical sarcocyst. Arai (1925) fed mice with spores of *S. tenella* of sheep. He noted that, during the first three hours, the spores could be found in all parts of the intestine, but only those in the upper parts of the small intestine were unchanged in appearance. Those occurring lower down were evidently in a degenerate or dying condition. The unchanged spores high up in the intestine during the three hours following the feed applied themselves to the surface of the epithelium, passed in between the cells, but not into them, and appeared finally in the subepithelial tissue. Within six hours of feeding all spores had disappeared from the lumen of the intestine, while they could not be detected in the subepithelial tissues later than four hours after feeding. On one occasion a spore was found in blood taken from the tail five hours after feeding, and on two occasions in the heart blood six hours after feeding. Between this time and the appearance of young parasites in the muscle fibres thirty-five to fifty days later the spores were not traced. It seems to be an undoubted fact that in the case of the mouse the spores penetrate the intestinal wall, but it is an exceedingly difficult matter to trace such

minute objects in their wanderings, and at the same time to exclude every possibility of confusing them with portions of cell nuclei or other small bodies in the tissues.

Very little is known about the development of Sarcosporidia in other animals. Bertram (1892) described young stages of the *S. tenella* of sheep. The smallest forms consisted of elongate cytoplasmic bodies 4 to 5 microns in length with a single nucleus. Older forms possessed a definite membrane, and consisted of round or oval cells lying in spaces in a matrix. By multiplication of the cells the spaces are enlarged and the matrix between the spaces becomes the walls or septa of the chambers. When multiplication has produced the requisite number of cells in a chamber, they become transferred into typical spores.

Pathogenicity.—In the majority of cases, even when fairly heavy infections exist, there is little evidence that the host is adversely affected. Sheep have sometimes died with very heavy infections, and death has been attributed to the Sarcosporidia. Creech (1922) has described extensive muscular degeneration in pigs caused by these parasites. In experimental mice the animals sometimes die, apparently as a result of intense infection. A curious feature of the Sarcosporidia is that they appear to contain a substance which is highly toxic to animals. Pfeiffer (1890) showed that the parasites of sheep were highly toxic if injected into mice, rabbits, and even sheep. Kasparek (1895) also showed that subcutaneous injection of the sheep parasite killed mice and guinea-pigs. Laveran and Mesnil (1899) made aqueous or glycerine extracts, and found that the extract of 0·001 gram of fresh parasite when injected subcutaneously killed rabbits in five to ten hours. Rats, mice, sheep, and frogs were not affected. They named the toxin "sarcocystin." Similar experiments were conducted by Rievel and Behrens (1904) with the Sarcosporidia obtained from llamas. Teichmann (1910) used a dried extract of the sheep parasite, which killed rabbits when injected intravenously in a dose of 0·0002 gram dissolved in saline solution. Rats and guinea-pigs were refractory. It was shown by Teichmann and Braun (1911) that rabbits could be immunized against the toxin, and that the serum contained antibodies which could produce passive immunity in other animals.

Method of Infection.—Though it is easy to understand how infection will spread amongst animals, such as rats and mice, which eat flesh, it is difficult to see how this happens in the case of cattle and sheep, which are nearly always infected and are purely herbivorous in diet. It occasionally happens that in cattle the Sarcosporidia infect the skin, and recently Sergent, Ed. (1921), has had the experience of finding the spores in blood-films made from these animals after pricking the skin. He has raised the

question of the possibility of biting flies taking up spores from the skin Watson (1909) also drew attention to the occurrence of spores in blood films.

Sarcosporidia in Man.

According to Darling (1909, 1910*a*), who has recorded two cases of human sarcosporidiosis, the infection is rare in man. The organism

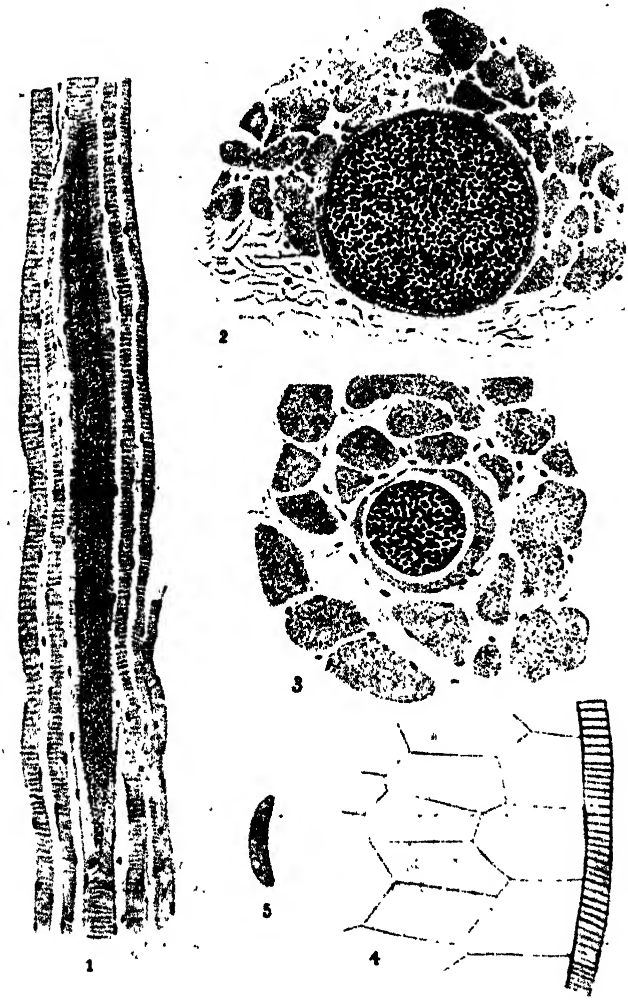


FIG. 330.—*Sarcocystis lindemanni* FROM THE MUSCLE FIBRES OF THE HUMAN LARYNX. (AFTER BARABAN AND ST. REMY, 1893.)

1. Longitudinal section of a muscle with a cyst *in situ* (× 300).
2-3. Transverse section of infested muscle fibres (× 300).
4. Portion of a section of a cyst from which the spores have dropped out, showing the septa (× 680). 5. Single spore (× 1,600).

described from man by Rivolta (1878) as *Gregarina lindemanni* is probably one of these parasites. Rosenberg (1892) saw certain structures, which are possibly Sarcosporidia, in the heart muscle of a man. He proposed to name the parasite *S. hominis*. A case regarded as authentic by Darling (1910*a*) was reported by Kartulis (1893). There appears to be no doubt about one described by Baraban and St. Remy (1894). In this case the infection occurred in the larynx, the muscle fibres of which were distended to about four times their normal thickness (Fig. 330). The first case described by Darling was in a negro. The parasites were discovered in portions of the biceps muscle, which had been removed owing to suspicious signs of trichinosis. The patient was actually suffering from typhoid fever, and it is concluded that the pains complained of in the muscles were actually due to the typhoid infection, and not to the Sarcosporidia. The largest parasites found had a length of 84 microns and a breadth of 27 microns, while the spores measured 4·25 by 1·75 microns. The second case described by Darling (1919) was in an East Indian who had died of malaria. Sections of the tongue revealed the parasites in the muscle fibres. Manifold (1924) described an infection of the muscle fibres of a human heart. The spores in this case were over 10 microns in length.

Sarcosporidia in Animals.

Though Miescher (1843) discovered the Sarcosporidia in the muscle fibres of the mouse, they were first named by Kühn (1865) *Synchytrium miescherianum*. As they evidently did not belong to this genus, Ray Lankester (1882) established the genus *Sarcocystis*, by which name they are now known. As already remarked, many forms have received distinctive names because of variations in size and their occurrence in different hosts. Alexeieff (1913*a*) justly remarks that there is no means of distinguishing the supposed species. He concludes that they all belong to the one species, *S. miescheriana* (Kühn, 1865). The following forms have been recorded:

Recorded Species of Sarcocystis.

MAMMALS:		
S. lindemanni	Rivolta, 1878	Man
S. muris	Blanchard, 1885	Rat, mouse
S. hirsuta	Moulé, 1887	Ox
S. cruzi	Hasselmann, 1923	Ox
S. blanchardi	Doflein, 1901	Buffalo
S. fusiformis	Railliet, 1897	Buffalo
S. siamensis	v. Listow, 1903	Buffalo
S. tenella bubali	Willey, Chalmers, and Philip, 1904	Buffalo
S. tenella	Railliet, 1886	Sheep
S. miescheriana	Kühn, 1865	Pig
S. bertrami	Doflein, 1901	Horse
S. hueti	Blanchard, 1885	Seal (*Otaria californica*)
S. leporum	Crawley, 1914	Rabbit

MAMMALS—*Continued:*		
S. pitymysi	Splendore, 1918	Field vole (*Pitymys savii*)
S. sp.	Krause, 1863	Dog, cat
S. cameli	Mason, 1910	Camel
S. richardi	Hadwen, 1922	Seal (*Phoca richardi*)
S. sp.	Hadwen, 1922	Reindeer
S. sp.	Hadwen, 1922	Caribou
S. aucheniæ	Brumpt, 1913	Llama
S. gazellæ	Balfour, 1913	*Gazella rufifrons*
S. kortei	Castellani and Chalmers, 1909	*Macacus rhesus*
S. gracilis	Von Ratz, 1908	Deer
S. moulei	Neveu-Lemaire, 1912	Goat
S. cuniculi	Brumpt, 1913	Rabbit
S. darlingi	Brumpt, 1913	Opossum
S. bubalis	Dogiel, 1916	*Bubalis cokei*
S. woodhousei	Dogiel, 1916	*Gazella granti*
BIRDS:		
S. rileyi	Stiles, 1893	Duck
S. horvathi	Von Ratz, 1908	Chicken
S. falcatula	Stiles, 1893	*Habia ludoviciana*
S. sp.	Barrows, 1883	*Parula pitiayumi*
S. turdi	Brumpt, 1913	*Merula merula*
S. colii	Fantham, 1913	*Colius erythromelon*
S. setophagæ	Crawley, 1914	*Setophaga ruticilla*
S. aramidis	Splendore, 1907	*Aramides saracura*
S. ammodromi	Splendore, 1907	*Ammodromus manimbe*
LIZARDS:		
S. platydactyli	Bertram, 1892	Gecko (*Platydactylus facetanus*)
	Chatton and Avel, 1923	Gecko (*Tarentola mauritanica*)
S. gongyli	Trinci, 1911	*Gongylus ocellatus*

Globidium Flesch, 1884.

These parasites, which are probably related to the Sarcosporidia, have the form of spherical cysts up to 5 millimetres in diameter embedded in the mucosa of the alimentary canal or skin of mammals (Fig. 331, I). Each is enclosed by a membranous capsule, and when fully grown consists of groups of spores which resemble those of the Sarcosporidia.

Flesch (1883) was the first to discover one of these parasites in the small intestine of the horse. He (1884, 1884*a*) gave it the name *Globidium leuckarti*. This species was rediscovered by Hobmaier (1922), and has been studied by Kupke (1923). Blanchard (1885) saw a similar parasite in a kangaroo and, believing it to be related to the Sarcosporidia, named it *Sarcocystis mucosæ*. Moussu and Marotel (1902) observed a form in the sheep, and regarded it as a developmental stage of the coccidium, *Eimeria faurei*. It was studied by Gilruth (1910), and in the same year by Chatton (1910), who named it *Gastrocystis gilruthi*. A similar parasite was discovered in the subcutaneous tissue and muscles of a cow by Besnoit and Robin (1912), according to whom it was named *S. besnoiti* by Marotel in 1912. Franco and Borges (1916), who studied this organism, came to the conclusion

that it differed sufficiently from other members of the genera *Sarcocystis* and *Gastrocystis* (*Globidium*) to justify the creation of a new genus, ***Besnoitia.*** It is evidently very similar to the members of the genus ***Globidium,*** in which it seems better to retain it at present as *G.* ***besnoiti.***

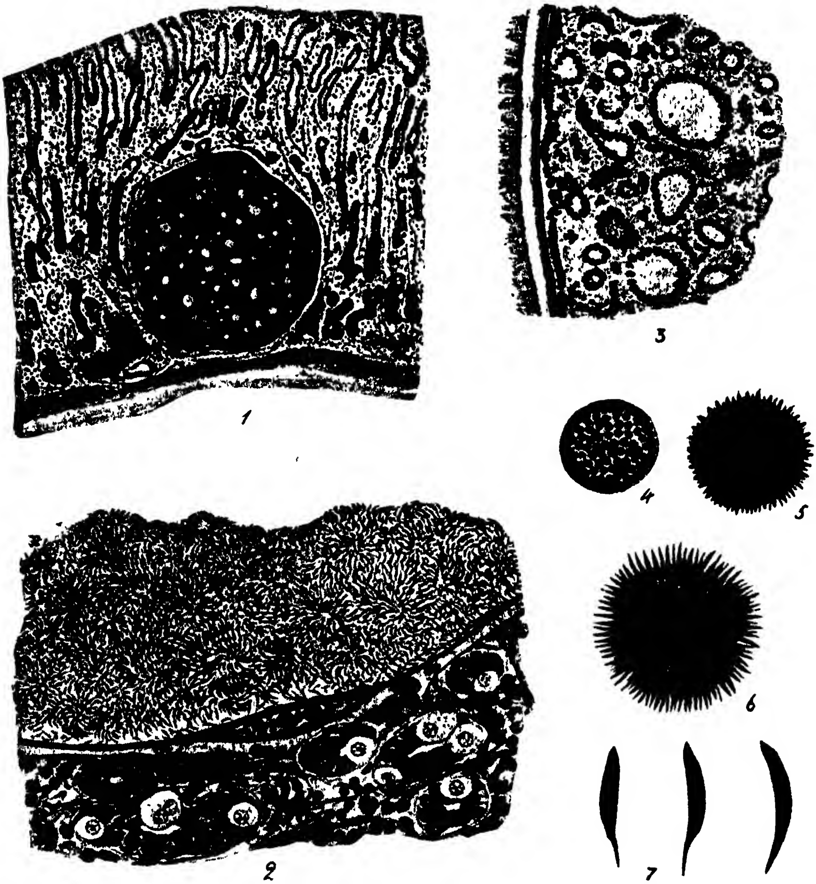


FIG. 331.—*Globidium gilruthi* FROM THE MUCOSA OF THE STOMACH OF SHEEP AND GOATS. (AFTER CHATTON, 1910.)

1. Section of mucosa showing cyst (×*ca.* 100).
2. Section of portion of immature cyst more highly magnified (×*ca.* 250).
3. Section of portion of mature cyst filled with spores (×*ca.* 500).
4-6. Method of development of spores from multinucleated cytoplasmic bodies (×*ca.* 500).
7. Individual spores (×*ca.* 2,000).

Gilruth and Bull (1912) described a series of parasites which they found in the intestinal mucosa of the kangaroo, wallaby, and wombat of Australia. In the kangaroo (*Macropus* sp.) there were large and small cysts, which they supposed belonged to different organisms The larger was named

Ileocystis macropodis, and the smaller one *Lymphocystis macropodis*. A large one in the wombat (*Phascolomys latifrons*) was called *I. wombati*. Believing the form in the wallaby (*Petrogale* sp.) to be a sarcosporidian, they gave it the name *S. macropodis*. The large cysts, which were named *I. wombati*, had thick walls, and reached a diameter of about 93 to 113 microns. In structure they resembled *G. gilruthi*. The smaller cysts, named *L. macropodis*, occurred in the connective tissues of the mucosa in large numbers. When mature, they had a diameter of about 8·4 microns and were filled with spores. The cyst wall was merely a membrane, which appeared to consist of the remains of a mononuclear cell, the nucleus of which could be detected as a flattened structure at one side. It is possible that *L. macropodis* is really a small form of *I. macropodis*, in which, however, free spores were not seen, though stages showing many nuclei and what appeared to be commencing spore formation occurred. The parasite called *S. macropodis* was also in the mucosa, measured 150 to 700 microns in diameter, and was filled with spores. It appears to be a species of *Globidium*, though Chatton (1912*c*) has suggested placing it in a new genus, *Haplogastrocystis*. Recently the writer and Scott (1925) and Triffitt (1926) have seen in the wallaby (*M. bennetti*) parasites like *I. macropodis* and *L. macropodis*. It was not possible to determine whether the smaller form was actually of the same species as the larger one, though this would seem not improbable. The whole of the connective tissue of the mucosa was filled with the smaller parasite, while numerous free spores were scattered between the cells. In addition, the muscle fibres of the intestine contained elongate vacuolic spaces filled with spores, which appeared very similar to those of the parasite of the *Lymphocystis* type. Whether this parasite, again, is a species of *Sarcocystis* or is another stage of *Lymphocystis* could not be decided. If three parasites are represented, then in this portion of the intestine there occurred four distinct species, as an *Eimeria* was present in the epithelium. It is possible that the parasite described by Blanchard (1885) and named *S. mucosæ* is identical with *I. macropodis*. Cunha and Torres (1924) record a species (*G. tatusi*) found by them in the armadillo.

The structure of the cysts of *G. gilruthi* of the sheep and goat was described in detail by Chatton (1910). The cysts have a diameter of 200 to 500 microns, and are situated within little opalescent elevations of the mucosa (Fig. 331, *1*). Each is enclosed by a definite wall, which has concentric striations. At one place in the wall there is a large flattened nucleus, which may be 80 microns in length by 10 in breadth (Fig. 331, *2*). According to Chatton, the cyst wall represents the remains of a very much hypertrophied and altered cell which may be connected by a kind of neck with the connective tissue. Within the mature cyst is a mass of spores, each of which measures 10 by 1·5 microns (Fig. 331, *7*). One end is blunt

and the other pointed, and, as in the spores of the Sarcosporidia, there is a nucleus near the blunt end, while the other end is clear and hyaline. The cytoplasm contains granules, one of which lies between the nucleus and clear pointed end and is distinctly larger than the others. In cysts which have not completed their development there occur spherical bodies with numerous nuclei arranged over the surface (Fig. 331, 4). Portions of cytoplasm, each with one of the nuclei, then grow out from the surface as pointed buds, which gradually assume the character of spores. The latter remain attached to the residue of cytoplasm till they break loose and are scattered within the cyst (Fig. 331, 5, 6).

FIG. 332. — *Globidium leuckarti* FROM INTESTINAL MUCOSA OF THE HORSE (× 600). (AFTER KUPKE, 1923.)

Section showing opening at the pole and still undivided contents.

The infection is very common in the abomasum. In the majority of animals the infection is small, but it is sometimes heavy. As the cysts, when mature, rupture into the stomach, in heavy infections hæmorrhages may be caused and serious symptoms follow. Triffitt (1925) has found *G. gilruthi* in as many as 92 per cent. of British sheep.

According to Franco and Borges (1916), infections of the skin with *G. besnoiti* may occur, as also of the connective tissue and fasciæ of the muscles. In the latter case, the muscles may appear studded over with white nodules due to the presence of the parasite, so that the flesh has to be condemned as unfit for food.

Kupke (1923) has studied *G. leuckarti* of the intestine of the horse. He finds that the parasite is embedded in a very much hypertrophied cell, the nucleus of which can often be detected lying at one side (Fig. 332). The parasite itself is an ovoid body consisting of a thick capsule which, in serial sections, can be seen to possess a definite pore at one end. The mass of cytoplasm within the capsule becomes multinucleate and divides into a number of separate bodies, each of which develops a number of nuclei. Presumably, each body gives rise to a cluster of spores.

The various species of *Globidium* agree fairly closely with that of the sheep described above. Some of the forms have been described as species of *Sarcocystis*, to which they undoubtedly bear some resemblance. Hobmaier (1922) has expressed it as his belief that the parasites are really fungi, and not Protozoa. It seems probable that the organisms are related to *Rhinosporidium*, described below (Fig. 336).

The following species have been described:

G. leuckarti	Flesch, 1883	Horse
G. gilruthi	Chatton, 1910	Sheep and goat
G. besnoiti	Marotel, 1912	Cattle
G. mucosæ	Blanchard, 1885	Kangaroo (*Macropus penicillatus*)
G. (*Ileocystis*) *macropodis* *G.* sp. (*Lymphocystis macropodis*)	Gilruth and Bull, 1912	Kangaroo (*Macropus* sp.)
G. sp. (*Sarcocystis macropodis*)	Gilruth and Bull, 1912	Wallaby (*Petrogale* sp.)
G. wombati	Gilruth and Bull, 1912	Wombat (*Phascolomys latifrons*)
G. tatusi	Cunha and Torres, 1923	Armadillo

Under the name of *Fibrocystis tarandi*, Hadwen (1922) describes certain cysts which occur in the fibrous tissue, especially that covering the tendons and the periosteum, of the reindeer and caribou. The cysts have a diameter of 100 to 450 microns, and consist of three layers enclosing numerous spores. In the reindeer it gives rise to a condition known as "corn-meal disease," on account of the gritty feel of the affected parts. When in the periosteum, the cysts cause the bone to atrophy, so that it becomes pitted. Both the reindeer and the caribou suffer from sarcosporidiosis of the muscles, and though the cysts of *F. tarandi* differ structurally from the Sarcosporiida, which resemble those of sheep, this difference may be due to their development in the fibrous tissue.

Haplosporidia Lühe, 1900.

Under this heading are included a number of parasites which in many respects resemble the Microsporidiida. They are found in aquatic invertebrates and fish, and occur as small uninucleated amœboid bodies or as multinucleate plasmodia. They float freely in the body-cavity fluid of the invertebrates, or infest the cells such as those of the intestine. In fish they attack the gills or other tissues, giving rise to white nodules. After growth and multiplication have taken place spores are produced, but these are not provided with polar capsules. The spore is spherical or ovoid, and the surface may be variously marked with ridges or tubercles. In some cases it is provided with a tail-like process. The genus *Bertramia*, established by Caullery and Mesnil (1899), includes forms which are parasitic in the body-cavity fluids of aquatic worms and rotifers. The minute uninucleate body develops into a cylindrical or sausage-shaped plasmodium containing many nuclei. It finally divides into a number of uninucleate forms. Roughly spherical and irregularly marked spores are produced. The genus *Ichthyosporidium* Caullery and Mesnil (1905) includes several species which infect fish. As in the case of Microsporidiida, white nodules are produced in the tissues or on the gills, and these are seen to

consist of an encapsuled multinucleate plasmodium in which occur ovoid spores. They may be confused with Microsporidiida, a mistake which was made by Thélohan (1895), who placed *I. giganteum* in the genus *Glugea.* The members of the genus *Haplosporidium* Caullery and Mesnil (1899) are parasitic in marine annelids. They give rise to spherical cysts, in which the plasmodium breaks up into a number of uninucleate bodies, each of which divides into four to form four ovoid spores. Each spore has one end flattened. Granata (1914) described in detail the development of *H. limnodrili* parasitic in the intestinal epithelium of *Limnodrilus udekemianus*

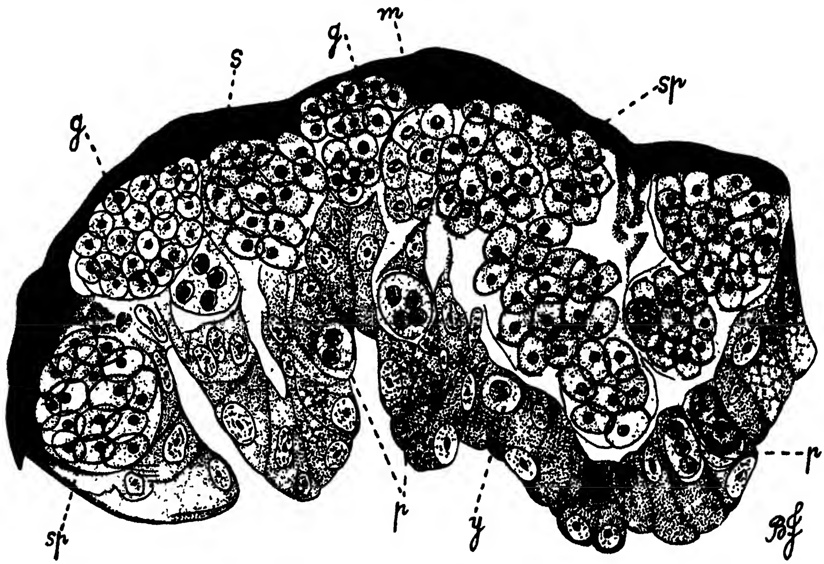


FIG. 333.—SECTION OF INTESTINE OF *Limnodrilus udekemianus* INFECTED WITH *Haplosporidium limnodrili* GRANATA, 1914 (× 750). (AFTER GRANATA, 1914.)

y, Young form with single nucleus; *p*, older forms with two or more nuclei; *s*, form with four nuclei (schizont); *m*, young forms resulting from division of schizont; *g*, forms which give rise to spores, gametes, or zygotes; *sp*, spores.

(Fig. 333). The genus *Urosporidium* Caullery and Mesnil (1905) is closely related to *Haplosporidium.* The spore is provided with a long caudal process. The genus *Cœlosporidium* Mesnil and Marchoux, 1897, was established for certain parasites of the kidneys of Crustacea. Crawley (1905) placed in this genus a parasite of the Malpighian tubes of the cockroach which had been taken for a microsporidian (*Nosema periplanetæ*) by Lutz and Splendore (1903). It occurs as amœboid bodies and ovoid spores in the cytoplasm of the cells. Another genus is *Serumsporidium* Pfeiffer, 1895, which includes parasites of the cœlomic fluid of Crustacea. They have been studied by Nöller (1920*b*) and Stempell (1921). Nöller

described *S. melusinæ* from the body cavity of *Simulium reptans.* The smallest uninucleated forms measured 5 to 7 by 3 to 4 microns. This develops into a multinucleate plasmodium, which becomes enclosed in a cyst from 25 to 70 microns in diameter. Within the cyst the plasmodium divides into a large number of the uninucleate forms. Stempell, describing the parasites from the crustacean *Herpetocypris strigata,* has noted the formation of spores which differ as regards their shape, external markings, and contents. He recognizes several genera.

A parasite in the form of small uninucleate bodies and multinucleate spheres was discovered by Calkins (1900) in the lymphatic system of trout which were dying in an epidemic. Calkins gave the name *Lymphosporidium truttæ* to the parasite. The parasite which Woodcock (1904) discovered in plaice and flounders in the form of small white nodules on the

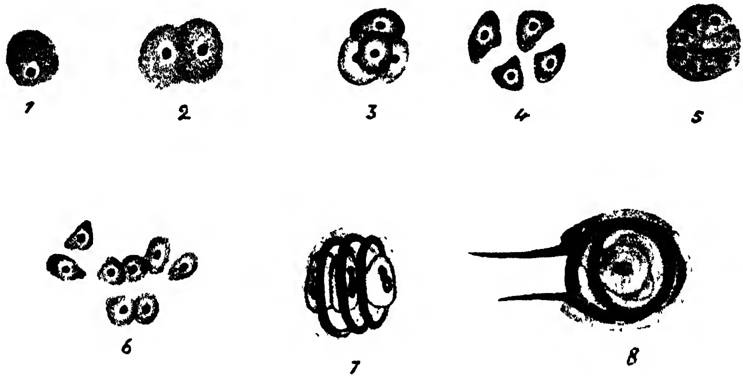


FIG. 334.—*Helicosporidium parasiticum* (× ABOUT 3,000). (AFTER KEILIN, 1921.)
1-6. Stages in schizogony.
7. Mature spores with coiled filament and three amœboid bodies.
8. Ruptured spore showing escape of filament.

surface of the internal organs was named by him *Lymphocystis johnstonei.* The nature of the organism is not known, some thinking it to belong to the Microsporidiida.

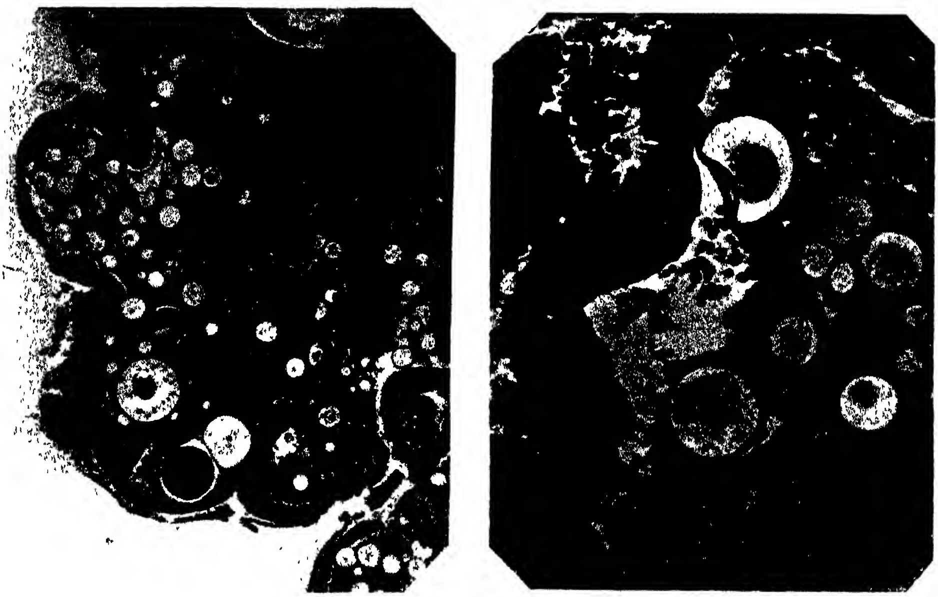
A curious parasite, *Helicosporidium parasiticum,* has been described by Keilin (1921) from the larvæ of *Dasyhelea obscura,* a ceratopogon (Fig. 334) The body spaces of the larvæ are invaded by small round cells, which multiply by schizogony. The schizonts are 4 microns in diameter, and give rise to four to eight merozoites. The remarkable feature of the parasite is its spore, which is a spherical body 5 to 6 microns in diameter. The capsule encloses four cells, three of which are amœboid, while one develops into a long coiled and resistant filament which appears to be free within the cyst. When the host dies, the spores rupture apparently by aid of

the spirally coiled filament which is discharged and remains free in the medium. It is 60 to 65 microns in length, and the nucleus of the cell from which it was derived is still present 15 microns from one extremity. The spores do not resemble those of any known microsporidian, for the filament is not developed in a polar capsule, and is a much stouter structure than those discharged from the spores of Microsporidiida.

It will be evident that the parasites which have been considered under the heading Haplosporidia form a very heterogeneous group. It seems highly probable that some of them, at least, are really fungi.

Rhinosporidium Minchin and Fantham, 1905.

Under this heading are included certain organisms which give rise to polypi, especially in the nose, of human beings and horses. They are



A B

FIG. 335.—SECTION OF NASAL POLYP FROM A CASE OF INFECTION WITH *Rhinosporidium seeberi* (A, × 60; B, × 260).

(Microphotographs of sections of tissues given to the writer by Professor J. H. Ashworth.)

undoubtedly vegetable parasites, as conclusively demonstrated by Ashworth (1923), but they are considered here, as for a long time they were regarded as Protozoa. Their structure and development appear to throw light on the forms described above, the Protozoon affinities of which are extremely doubtful.

Rhinosporidium seeberi (Wernicke, 1903).—This organism was first seen by Seeber (1900) in a nasal polyp in South America (Figs. 335, 336). As pointed out by Ashworth (1923), it was referred to by Belou (1903) as

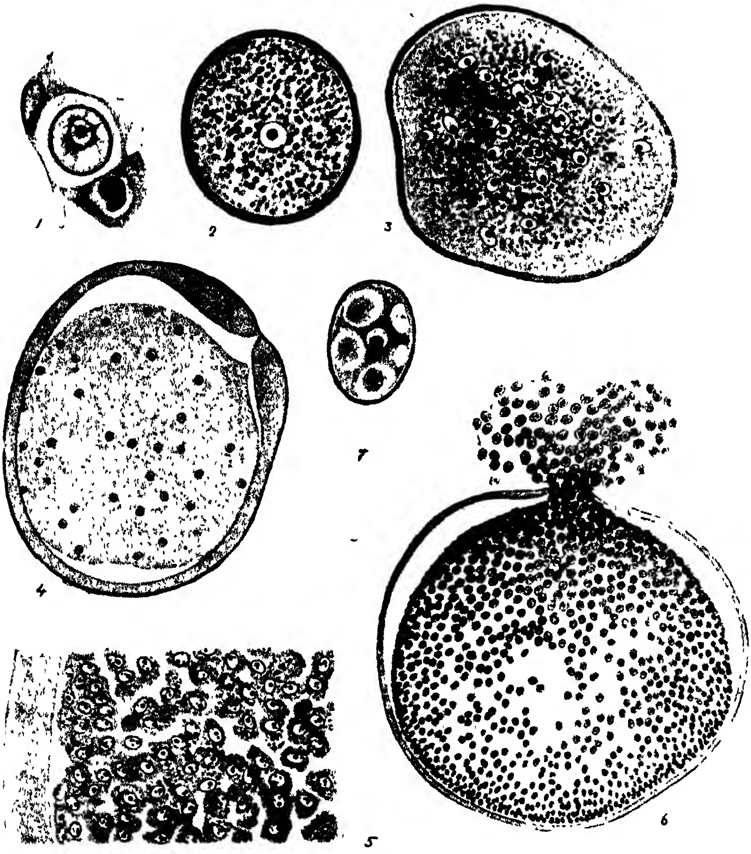


FIG. 336.—STAGES IN THE DEVELOPMENT OF *Rhinosporidium seeberi* FROM NASAL POLYP OF MAN. (AFTER ASHWORTH, FROM *Trans. Roy. Soc., Edin.*, liii., 1923.)

1. Very early stage 6 μ in diameter lying between connective tissue cells ($\times$ 1,600).
2. Later stage 65 μ in diameter with single nucleus ($\times$ 400).
3. Section of later stage with sixty-four nuclei ($\times$ 400).
4. Section of stage with about 500 nuclei. The envelope is composed of a thin chitinous external layer and a thick inner cellulose layer. The position of the future pore is indicated by a depression in the cellulose layer ($\times$ 400).
5. Section of stage in which the contents of sporangium has subdivided into about 4,000 nucleated cells ($\times$ 800). 6. Discharge of mature spores through pore of sporangium ($\times$ 200).
7. Section of a spore (10 $\times$ 7 μ), showing nucleus with karyosome and cytoplasm containing vacuoles, three of which include refringent spherules.

Coccidium seeberia Wernicke, 1900. Minchin and Fantham (1905) named it *R. kinealyi*, but undoubtedly Wernicke's name, *R. seeberi*, has priority, as pointed out by Hartmann (1921). Minchin and Fantham regarded the

organism as a Protozoon belonging to the Haplosporidia, but Ashworth has shown conclusively that it is a vegetable parasite allied to the fungi. The organism produces nasal polypi in man (Fig. 335), and has also been seen in polypi of the conjunctiva, lacrimal sac, and ear, in a papilloma of the penis, and in the uvula. According to Ashworth (1923), who has given a complete account of the organism, the younger forms are spherical bodies about 6 microns in diameter embedded in the cytoplasm of connective tissue cells. Each has a capsule enclosing a mass of cytoplasm with a single nucleus and a number of deeply-staining granules of reserve food material (Fig. 336, I). Growth takes place, nuclear multiplication by mitosis occurs, and the cytoplasm becomes charged with numerous food granules. Eventually, the central cytoplasm segments into uninucleate masses, and this process spreads towards the periphery of the cyst till the contents are completely divided. Multiplication by fission of the separate masses may occur. Eventually, each separate mass becomes enclosed in a membrane. Meanwhile, the parent cyst, which now has a diameter of 250 to 300 microns, develops a thick lining composed of cellulose and a definite pore forms at one point. Through this pore the daughter cysts are discharged to spread the infection to neighbouring tissues Each daughter cyst is taken up by a mononuclear cell and commences to grow. The infection, which is of a very chronic type, has been recorded from India, Cochin-China, Ceylon, Argentina, and North America.

Zschokke (1913) described *R. equi* from the nasal septum of a horse in South Africa. According to Hartmann (1921), Frey and Hartmann arrived at conclusions regarding the nature and development of the organism similar to those put forward by Ashworth for *R. seeberi*. That there is any specific difference between the human and equine form seems doubtful.

END OF VOL. I.